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(54) Title: METHODS OF STIMULATING PHAGOCYTOSIS

(57) Abstract

The present invention relates, in general, to methods of stimulating phagocytosis and thereby combating infection and/or modulating immune complex disease, in particular, to methods of modulating the number and type of Fc receptors present on cells that normally possess such receptors, including monocytes and macrophages, as well as on cells that normally do not possess Fc receptors, such as fibroblasts, and to compounds and compositions suitable for use in such methods.

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METHODS OF STIMULATING PHAGOCYTOSIS

This is a continuation-in-part of Application No.
08/129,391, filed September 30, 1993, the contents of
which are incorporated herein by reference.

TECHNICAL FIELD

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The present invention relates, in general, to
methods of stimulating phagocytosis and thereby
combating infection and/or modulating immune complex
disease, in particular, to methods of modulating the
number and type of Fc receptors present on cells that

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normally possess such receptors, including monocytes
and macrophages, as well as on cells that normally do
not possess Fc receptors, such as fibroblasts, and to
compounds and compositions suitable for use in such

15 methods.

BACKGROUND

Mononuclear phagocytes (blood monocytes and tissue
macrophages) have cell surface receptors for the Fc
domain of IgG antibody. These receptors (FCγR) mediate

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humoral immune effector functions including
phagocytosis, clearance of immune complexes and
antibody-dependent cell cytotoxicity. Three classes of
Fcγ receptors have been identified on human cells and
characterized on the basis of size, primary structure,

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binding affinity for IgG subclasses, and recognition by

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monoclonal antibodies: FcγRI (CD64), FcγRII (CD32), and
FcγRIII (CD16). FcγRI is a high affinity receptor,
expressed on resting mononuclear phagocytes and
stimulated neutrophils. FcγRII and FcγRIII are low
affinity receptors found on a range of hematopoietic
cells, including monocytes and macrophages.
Macrophages express all three receptor classes while
monocytes express primarily FcγRI and FcγRII.

All three classes of human Fcγ receptors have been
isolated and cloned (Allen and Seed, Science 243:378
(1989); Hibbs et al, Proc. Natl. Acad. Sci. USA 85:2240
(1988); and J. Exp. Med. 166:1668 (1987)). At least
two genes code for the FcγRI class of receptors (van de
Winkle et al, FASEB J. 5:A964 (1991)), three genes code
for the FcγRII class (designated FcγRIIA, FcγRIIB and
FcγRIIC) (Brooks et al, J. Exp. Med. 170:369 (1989);
Stuart et al, EMBO J. 8:3657 (1989); Qui et al, Science
248:732 (1990)) and two genes code for the FcγRIII
receptor class (Simmons and Seed, Nature 333:568
(1988)).

Macrophage Fcγ receptors participate in the
clearance of IgG-coated particulate and soluble
antigens, including IgG-coated microorganisms, and
thereby remove potentially dangerous foreign organisms.
Due to their importance in host defense, functional
integrity of Fcγ receptors has been studied in
connection with various disease states, including
autoimmune disorders (Frank et al, Ann. Intern. Med.
98:206 (1983); Kimberley and Ralph, Am. J. Med. 74:481
(1983)) and end-stage renal disease (Ruiz et al, N.

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Engl. J. Med. 322:717 (1990)). Macrophage Fcγ receptor
function has been found to be decreased in patients
with certain HLA haplotypes and in patients with the
immune disorders systemic lupus erythematosus,
Sjogren's syndrome and dermatitis herpetiformis (this
observation was attributed to occupation of these
receptors on the macrophages by immune complexes). In
end-stage renal disease, macrophage Fcγ receptor
function has been found to be impaired and this
impairment is believed to contribute to the observed
immunodepression among such patients.

Various diseases, non-bacterial in origin, are
associated with a high incidence of complications due
to infection. Examples of such diseases include the
above-noted end-stage renal disease (Goldblum and Reed,
Ann. Intern. Med. 93:597 (1980); Lahnborg et al,
Transplantation 28:111 (1979); Drivas et al, Invest.
Urol. 17:241 (1979); Keane and Raij, In: Drukkar et al
eds. Replacement of Renal Function by Dialysis, 2nd
ed., pp. 646-58 (1983)), acquired immunodeficiency
syndrome (AIDS) (Bender et al, J. Infect. Disease
152:409 (1985), Smith et al, J. Clin. Invest. 74:2121
(1984)), liver disease (Rimola, In: McIntyre et al eds
Oxford Textbook of Clinical Hepatology, pp. 1272-84
(1991)) and diseases of the lung, including cystic
fibrosis (Gomez and Schreiber, unpublished
observations) and acute respiratory distress syndrome
(ARDS) (Rossman et al, Clin. Res. 41:251A (1993)).
Defective Fcγ receptor-dependent clearance has been
observed in certain of these diseases. Thus, there is

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a clear need for methods that can be used to correct
defective Fcγ receptor function and/or enhance
functional Fc receptor expression and thereby stimulate
host defense. The present invention provides such
5   methods and compounds and compositions suitable for use
therein.

OBJECTS AND SUMMARY OF THE INVENTION

It is a general object of the present invention to
provide a method of combating infection by stimulating
10   phagocytosis.
It is the specific object of the invention to
provide a method of stimulating phagocytosis by
modulating the number and type of Fc receptors present
on cells that normally possess such receptors,
15   including monocytes and macrophages. In addition, it
is a specific object of the invention to provide a
method of combating infection by rendering cells
phagocytic that do not normally possess that function,
such as fibroblasts or epithelial or endothelial cells
20   not normally expressing Fcγ receptors.
It is a further object of the invention to provide
constructs suitable for use in gene therapy protocols
that encode Fc receptors, and cells transformed
therewith.
25   In one embodiment, the present invention relates
to a method of increasing the phagocytic potential of
cells present in a mammal that comprises introducing
into the cells a DNA molecule coding for an Fc

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receptor. The introduction is effected under
conditions such that the DNA molecule is expressed, the
Fc receptor produced, and the phagocytic potential of
the cells thereby increased.

5   In a further embodiment, the present invention
relates to a method of increasing the phagocytic
potential of cells of a mammal that comprises:

    i)   removing cells from the mammal,

    ii)   introducing into the cells a DNA molecule
10 encoding an Fc receptor, and

    iii) reintroducing the cells into the.mammal
under conditions such that the DNA molecule is
expressed, the Fc receptor produced, and the phagocytic
potential of the cells thereby increased. One skilled
15 in the art will appreciate that steps (i)-(iii) can be
carried out using methodologies known in the art.

        In other embodiments, the present invention
relates to a liposome comprising a DNA molecule
encoding an Fc receptor, a bacterium comprising a DNA
20 molecule encoding an Fc receptor, a T cell comprising
an exogenous DNA sequence encoding an Fc receptor, and
a B cell comprising an exogenous DNA sequence encoding
an Fc receptor.

        In yet another embodiment, the present invention
25 relates to a DNA construct encoding an Fc receptor
comprising domains, or functional portions thereof,
from at least two of FcγRI, FcγRII and FcγRIII, wherein
the domains, or portions thereof, are such that the
receptor renders cells phagocytic that comprise same.
30 The invention also relates to the encoded Fc receptor.

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In a further embodiment, the present invention relates to a method of treating an infection comprising administering to a mammal in need of such treatment a DNA molecule encoding an Fc receptor. The

5 administration is effected under conditions such that the DNA molecule is expressed in cells of the mammal, the Fc receptor produced, and the phagocytic potential of the cells thereby increased. The resulting cells phagocytose IgG-coated particles causing the infection,

10 or IgG-containing soluble immune complexes derived from the infection.

Further objects and advantages of the present invention will be clear from the description that follows.

15 **BRIEF DESCRIPTION OF THE DRAWINGS**

FIGURES 1A and 1B - A) Biotinylation of D58 (Src+) and SAR6 (Src-) cells infected with FcγRIIA. Immunoprecipitation with anti-FcγRII mAb IV.3 demonstrates the 40 kD FcγRIIA protein in the membrane

20 of FcγRIIA-infected cells (lanes 2 and 4). No receptor is present in the sham-infected cells (lanes 1 and 3). B) Phosphorylation of FcγRIIA on tyrosine after receptor crosslinking in FcγRIIA-infected D58 and SAR6 cells. Phosphotyrosine containing proteins were

25 immunoprecipitated from cell lysates with and without FcγRIIA stimulating (+EA and -EA). Induction of the tyrosine phosphorylated 40 KD receptor is seen in lanes 6 and 8.

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FIGURES 2A and 2B - Fluorescence histograms of (A)
D58 and (B) SAR6 cells infected with FcγRIIA. The
dotted line represents cells stained with an isotype
control mAB and the solid lines represent cells stained
5   with anti-FcγRII.

FIGURES 3A and 3B - *In vitro* immune complex kinase
assay of Src related tyrosine kinases from FcγRIIA
infected D58 (Src+) (lanes 1-6) and SAR6 (Src-) cells
(lanes 7-12). FcγRIIA-infected and sham-infected cells
10   were lysed and cell lysates immunoprecipitated with the
antibodies indicated above each lane (RAM is the
rabbit-anti-mouse control, IV.3 is anti-FcγRII mAb, Src
and Fyn are mAbs specific for these kinases). Immune
complexes were exposed to $[\gamma^{32}P]ATP$ to allow
15   autophosphorylation of the kinases and phosphorylation
of FcγRIIA. The positions of the phosphorylated Src,
Fyn and FcγRIIA proteins are indicated by the open
squares, stars and arrows, respectively. Lanes 2 and
8, representing immunoprecipitates with Src antibody
20   alone, confirm the Src+ and Src- phenotypes of the D58
and SAR6 cell lines.

FIGURE 4 - Macrophage Fcγ-receptor-mediated
clearance of IgG-sensitized radiolabeled red cells in
patients with alcoholic cirrhosis of the liver (n=49),
25   non-cirrhotic alcoholic subjects (n=10) and healthy
volunteers. The middle three curves (means ± SEM)
represent values for clearance of IgG-sensitized red
cells in these 79 subjects; the upper pair of curves,

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the clearance of unsensitized autologous red cells in
five patients and five controls; and the lower pair of
curves, the clearance of heat-damaged red cells (heated
for 30 minutes at 56°C) in five patients and five
5   controls.

        FIGURE 5 - Macrophage Fcγ-receptor-mediated
clearance of IgG-sensitized radiolabeled red cells in
patients with alcoholic cirrhosis of the liver (n=49),
and healthy volunteers (n=20). The four middle curves
10   (means ± SEM) represent values for clearance in these
69 subjects: patients with mildly decompensated
alcoholic cirrhosis of the liver (cirrhosis I, n=17),
patients with moderately decompensated alcoholic
cirrhosis of the liver (cirrhosis II, n=17), patients
15   with severely decompensated alcoholic cirrhosis of the
liver (patients III, n=15), and controls (n=20).

        FIGURE 6 - Macrophage Fcγ-receptor-mediated
clearance of IgG-coated red cells (as half-time) in
patients with alcoholic cirrhosis of the liver (n=49)
20   and in controls (n=20). The half-time was
significantly longer in the eleven patients in whom
severe infection developed during follow-up.

        FIGURE 7 - Recognition of human IgG(anti-RhD)-
coated red cells by monocytes from patients (n=49) and
25   controls (n=20). IgG-sensitized, ^{51}Cr-labeled (2 x 10)^7
erythrocytes were added to monolayers of monocytes, and
the percentage of red cells bound by monocytes was

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determined by measuring the radioactivity. Values are
means ± SEM.

    FIGURE 8 - Recognition of mouse IgG2b-coated red
cells by monocytes from patients (n=49) and controls
5   (n=20). IgG2b-sensitized erythrocytes were added to
monolayers of monocytes, and the percentage of
monocytes binding >3 RBC per cell was determined.
Values are means ± SEM.

    FIGURE 9 - Macrophage Fcγ-receptor-mediated
10   clearance in patients with circulating immune complexes
(n=7). The curves for these patients fell into the
range for the patient group.

    FIGURE 10 - Tyrosine phosphorylation in wild type
J32 and in mutant J32-3.2 transfectants.
15   Antiphosphotyrosine immunoblots were prepared following
immunoprecipitation of cell lysates with either anti-
phosphotyrosine antibody or anti-FcγRII antibody. The
40 kD FcγRII receptor is phosphorylated on tyrosine
following FcγRII activation.

20   FIGURES 11A and 11B - Fluorescence histograms of
J32/FcγRIIA and J32-3.2/FcγRIIA stable transfectants,
and FcγRIIA expressing clones. Flow cytometry was
employed with anti-FcγRII monoclonal antibody IV.3 or
with an isotype control (Indik et al, J. Clin. Invest.
25   88:1766 (1991)).

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FIGURES 12A and 12B - Phagocytosis of IgG coated erythrocytes by J32 and J32-3.2 transfectants. EA was prepared as described previously (Indik et al, J. Clin. Invest. 88:1766 (1991)), overlaid onto transfected or sham-transfected T-cells and incubated at 37°C for 30 minutes. Unbound EA was removed by washing with PBS and extracellular bound EA was removed by exposure to hypotonic buffer before staining with Wright-Geimsa.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of modulating the phagocytic potential of cells that are naturally phagocytic, such as macrophages, and to methods of rendering cells phagocytic that do not naturally possess that function. In so doing, the present invention provides innovative treatment regimens that can be used to combat infections associated with various disease states.

Drug Induced Enhancement of Fcγ Receptor Expression:

In one embodiment, the present invention relates to a method of enhancing Fcγ receptor expression on phagocytic cells of a mammal, including macrophages. The method comprises administering to the mammal an active agent, such as the cytokine interferon gamma (IFN-γ), an estrogen or estrogen analog, or a hematopoietic growth factor such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or

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macrophage colony stimulating factor (M-CSF). IFN-γ
has been shown to modulate the levels of FcγRI and
FcγRII apparently by increasing gene transcription.
Dexamethasone has been reported to influence this IFN-
5   γ-induced enhancement of transcription in a cell-
specific manner (Comber et al, Cell. Immunol. 145:324
(1992)). Estradiol and diethylstilbesterol have been
shown to facilitate clearance of IgG-coated cells
(Friedman et al, J. Clin. Invest. 75:162 (1985); Ruiz
10   et al, Clin. Res. 38:367A (1990)). GM-CSF has been
shown to selectively increase monocyte FcγRII
expression and function (Rossman et al, Exp. Hematol.
21:177 (1993)), and, similarly, M-CSF has been shown to
increase splenic macrophage Fcγ receptors and thereby
15   enhance the clearance of IgG-coated cells (Ruiz et al,
Clin. Res. 40:796A (1992)).

         One or more of the above-referenced active agents
can be combined with an appropriate carrier to form a
dosage form suitable for use in the method of the
20   present invention. The amount administered will vary
depending on the patient, the agent, the clinical
response sought and the route of administration.
Appropriate concentrations and dosage regimens can be
readily determined by one skilled in the art having
25   knowledge of these agents.

         The active agents can be formulated as capsules,
tablets, and the like, and as solutions and suspensions
suitable for intravenous or parenteral administration.
The agents can also be formulated as aerosols for
30   administration to the lung. Carriers used are

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pharmaceutically acceptable and depend on the dosage
form.

In vivo synthesis of the above active agents can
be effected, for example, at a particular site, by
5   introducing into cells of the patient sequences
encoding the agent in an appropriate vector (e.g. an
adenoviral or retroviral vector) preferably in
combination with an Fcγ receptor encoding sequence (see
below). In a preferred embodiment, the sequence
10   encoding the agent encodes M-CSF and the sequence
encoding the receptor encodes the γ chain of FcγRIII.
Such encoding sequences can also be administered, for
example, in liposomes, particularly where lung is the
target tissue.

15   Conditions amenable to treatment by the above-
noted active agents include those characterized by
reduced macrophage Fcγ receptor number or function, for
example, chronic renal failure, liver disease and
pulmonary disorders, including acute respiratory
20   distress syndrome (ARDS), AIDS and cystic fibrosis.
Such agents can be used in combination with one or more
of the therapeutic approaches described below to
enhance Fcγ receptor activity and thereby treat
infections that often accompany these conditions and
25   others.

Fcγ Receptor Gene Therapy:

In a further embodiment, the present invention
relates to the use of recombinant and gene therapy

protocols to modulate Fc receptor expression. As noted above, genes encoding all three classes of Fcγ receptors have been isolated and cloned. All three receptor classes, FcγRI, FcγRII and FcγRIII, consist of distinct domains corresponding to their location within the cell. The cDNA structure of the FcγRII class of receptors, for example, consists of a 5' untranslated region, sequences coding for a signal peptide region (S), an extracellular domain (EC), a transmembrane region (TM), an intracytoplasmic domain (C), and a 3' untranslated region (Schreiber et al, Clin. Immunol. Immunopath., 62:S66 (1992), Cassel et al, Molec. Immunol. 30:451 (1993)). Likewise, the predicted polypeptide sequence of FcγRI shows a hydrophobic signal sequence, a hydrophobic transmembrane region and a charged cytoplasmic domain, in addition to an extracellular region that consists of three immunoglobulin-like domains, two of which share homology with the other Fcγ receptors (Allen and Seed, Science 243:378 (1989); Schreiber et al, Clin. Immunol. Immunopath., 62:S66 (1992)). FcγRIIIA is a complex consisting of a single α chain and a homo- or hetero-dimer of associated γ and ζ chains (Letourneur et al, J. Immunol. 147:2652 (1991); Ra et al Nature (Lond.) 241:752 (1989); Park et al, Clin. Res. 41:324A (1993)). Both the γ and ζ chains mediate phagocytosis, the γ chain being more efficient (Park et al, Clin. Res. 41:324A (1993)). The extracellular domain of FcγRIII is closely homologous to that of FcγRI and FcγRII, however, the transmembrane domain of FcγIII terminates

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in a 200-220 residue hydrophobic domain followed by
four hydrophobic residues, one of which is charged
(Simmons and Seed, Nature 333:568-570 (1988)). FcγRIII
thus differs from FcγRI and FcγRII in that the latter

5   two have substantial intracellular cytoplasmic domains.
        FcγRI is unique among the three classes of human
Fcγ receptors not only in its high affinity for IgG but
also in the structure of its cytoplasmic domain.
Macrophage FcγRII and the γ chain of FcγRIII have

10   tyrosine residues in their cytoplasmic domains that are
required for phagocytosis. In contrast, FcγRI does not
contain tyrosine residues in its cytoplasmic domain
(Allen and Seed, Science 243:378 (1989)) and is not
phosphorylated on tyrosine. Further, FcγRI is unusual

15   among the Ig gene family of receptors in not requiring
its cytoplasmic domain for phagocytosis (Indik et al,
Clin. Res. 41:170A (1993)).
        Recombinant techniques make it possible to
manipulate the domains of naturally occurring receptors

20   and thereby design Fc receptors having specific
characteristics. The present invention contemplates
the use in gene therapy regimens of DNA sequences
encoding such selectively constructed receptors,
comprising domains from single or multiple Fcγ

25   receptors, to effect the production of receptors having
defined activities, both in cells that normally produce
Fcγ receptors and in cells that normally do not. In
the former case, the Fc receptor sequence introduced
into target cells can encode a protein essentially

30   identical to that normally produced by the cell.

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Alternatively, the sequence introduced can encode: i)
an Fc receptor protein that is functionally comparable
to, but structurally different from, the naturally
occurring receptor (e.g. a protein comprising only
5   functional portions of the domain(s) (for example, the
cytoplasmic domain) of the naturally occurring
receptor), or ii) a receptor protein that differs
functionally and structurally from the Fc receptor that
is normally present on the cell (e.g. a chimeric
10   receptor protein comprising a high affinity FcγRI
extracellular domain and transmembrane and cytoplasmic
domains from FcγRIIA or FcγRIIIA). The present
invention thus makes it possible to compensate for
deficiencies in the production of Fc receptors of a
15   particular functional type, which deficiencies may
occur in association with a particular disease state.
The invention also makes it possible to manipulate the
composition of the Fc receptor population of a
particular cell type. That is, a cell producing
20   predominantly high affinity receptors can be engineered
so as to produce predominantly low affinity Fc
receptors.

        The transmission of extracellular signals to
cellular targets by many surface receptors is dependent
25   upon interaction between cytoplasmic protein tyrosine
kinase and tyrosine-containing sequences in the
cytoplasmic domain of the receptor, or an associated
subunit. The *in vivo* kinase important for γ chain
mediated phagocytosis is Syk. The data presented in
30   Example XI make it clear that transfection or

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cotransfection of a Syk encoding sequence can be used
to enhance phagocytosis mediated by the γ chain (as
well as by the ξ chain, in a target cell. Various
constructs can be used for this purpose.

5   Equally important, the present invention makes it
possible to render cells phagocytic that do not
normally possess that function. Sequences encoding
naturally occurring Fcγ receptors or sequences encoding
non-naturally occurring Fc receptors, for example,
10   chimeric receptors that include entire domains, or
functional portions thereof, from two or more naturally
occurring Fcγ receptors, can be introduced into such
cells. The chimeric receptors can be designed so as to
take into account both the phagocytic potential of the
15   cells into which the encoding sequences are to be
introduced and the receptor domain properties suited
for achieving the desired therapeutic effect. While not
all cells are equally suitable as recipients for all Fc
receptor-encoding constructs, operability can be
20   readily assessed using *in vitro* model systems such as
those described by Indik et al (J. Clin. Invest.
88:1766 (1991) and Hunter et al, Clin. Res. 41:244A
(1993); see also Amigorena et al, Nature (Lond) 358:337
(1992); Park et al, Clin. Res. 41:324A (1993); Toijman
25   et al, Blood 79:1651 (1992); Kruskal et al, J. Exp.
Med. 176:1673 (1992); (see also Examples below)). This
embodiment of the invention may be particularly
advantageous since cells, such as fibroblasts, that are
rendered phagocytic may injest particles without
30   releasing significant quantities of superoxide radicals

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or toxic biologically active products. This is in
contrast to cells that are normally phagocytic, such as
macrophages. One skilled in the art will appreciate
that a reduction in the release of toxic products

5   results in a reduction in the possibility of
inflammation.

    Constructs:

        Chimeric Fc receptors suitable for use in the
present invention include those prepared as detailed in

10   the Examples below. For instance, single chain
chimeras of the α and γ chains of FcRIIIA can be
prepared. Sequences encoding such chimeras have been
introduced into COS-1 cells and the phagocytic
potential conferred examined. For example, a DNA

15   sequence er ncding the extracellular domain of the α
chain of   IIIA, the transmembrane domain of the γ
chain o.   RIIIA or FcγRI and the cytoplasmic domain
of the   in of FcγRIIIA has been transfected into
COS-.   (the transmembrane domain of the α chain of

20   FcγR   i be used in lieu of that of the γ chain,
thou.   naps not as effectively). Such chimeras
lisp.   nagocytic activity in the COS-1 assay system
hou   it at a level equivalent to the multichain form
f F   IIA. In spite of the reduced activity, single

25   chai   onstructs are clearly advantageous in view of
the difficulties inherent both in introducing into
target cells multiple sequences and in achieving proper
complexa ion of the encoded proteins.

        Fc chimeric receptors have also been prepared from

30   a combination of domains of FcγRII isoforms and from a

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combination of FcγRI and FcγRII domains. Specifically,
a chimeric receptor comprising the extracellular and
transmembrane domains of FcγRIIB2 and the cytoplasmic
domain of FcγRIIA has been shown to confer phagocytic
5   potential on host cells, thus demonstrating that the
FcγRIIB2 transmembrane domain is capable of
transmitting the phagocytic signal to the FcγRIIA
cytoplasmic domain (FcγRIIB receptors do not themselves
confer phagocytic potential). Similarly, a chimeric
10   receptor comprising the extracellular domain of FcγRI
and the transmembrane and cytoplasmic domains of
FcγRIIA has been shown to induce phagocytosis in host
cells. In contrast, chimeras comprising the
extracellular domain of FcγRI and the transmembrane
15   domain of FcγRI or FcγRIIA do not result in
phagocytosis when the cytoplasmic domain is from
FcγRIIA or FcγRI, respectively. However, chimeras
comprising the extracellular domain of FcγRI, the
transmembrane domain of FcγRI and the cytoplasmic
20   domain of the γ chain of FcγRIII, do result in
phagocytosis. It will be appreciated that chimeras
comprising the extracellular domain of FcγRI (and
appropriate transmembrane and cytoplasmic domains) can
be advantageous in view of the high binding affinity of
25   the FcγRI extracellular region.
        Chimeras in addition to those described above and
detailed below are contemplated. For example, the
cytoplasmic domain of FcγRIIA can be used in
combination with the extracellular domain of FcγRI and
30   the transmembrane domain of FcγRIIA. Further, the

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extracellular and transmembrane domains of FcγRI or
FcγRII can be used in combination with the cytoplasmic
domain of the γ chain of FcγRIII. Further, chimeras of
the invention can include the extracellular domain from

5   FcγRIIA, FcγRI or from the α chain of FcγRIII, the
transmembrane domain from FcγRIIA or from the α or γ
chain of FcγRIII, and the cytoplasmic domain of either
the γ chain of FcγRIII or FcγRIIA (e.g., i) the
extracellular and transmembrane domains of FcγRIIA, ii)

10   the extracellular domain of the α chain of FcγRIII and
the transmembrane domain of the γ chain of FcγRIII, or
iii) the extracellular domain of FcγRI and the
transmembrane domain of the α or γ chain of FcγRIII -
each with the cytoplasmic domain from either the γ

15   chain of FcγRIII or FcγRIIA (it is noted that
preliminary results suggest that certain chimeras
comprising the transmembrane domain of the α chain of
FcγRIII may not be operative).

       While chimeras of the invention can include the
20   entire extracellular, transmembrane and cytoplasmic
domains of the respective naturally occurring
receptors, such is not necessarily the case. Rather,
the chimeras can comprise only the functional
portion(s) of the respective domains. For example, in

25   the case of the cytoplasmic domain of FcγRIIA,
truncation at amino acid 303 (which results in deletion
of the terminal 8 amino acids but preservation of the
two tyrosine (Y282 and Y298)-containing core sequences
important in phagocytosis does not decrease

30   phagocytosis (Mitchell et al, Clin. Res. 41:1894A

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(1993)).   Truncation of the FcγRIIA cytoplasmic domain
at amino acid 268 or 280, however, results in receptors
lacking the tyrosines at positions 282 and 288, and
lacking phagocytic activity. These data are consistent

5   with the importance of tyrosine residues in the
cytoplasmic Fc receptor domain in transmission of the
cytoplasmic signal. In treatment regimens in which
suppression of phagocytic potential is advantageous
(for example, autoimmune diseases) these later mutants

10   or peptides derived from or mimicking these mutants can
be useful (see the commonly owned application entitled
"Method of Inhibiting Phagocytosis" filed concurrently
herewith, the entire disclosure of which is
incorporated herein by reference). It will be

15   appreciated, however, that when potentiation of
phagocytosis is sought, functionality of each of the
domains must be preserved. In this regard, it appears
that the second YX2L of the core sequence of the
cytoplasmic domain of FcγRIIA (E-X8-D-X2-Y-X2-L-X12-Y-

20   X2-L) and the γ chain of FcγRIIIA (D/E-X2,7-D/E-Y-X2-L-
X7-Y-X2-L) are particularly important for phagocytosis
(note also that the exon 3 domain of the γ chain of
FcγRIII that is 5' or amino terminal to the Y-X2-L
motif appears to play a role in phagocytosis since its

25   elimination diminishes phagocytosis by the γ subunit of
FcγRIIIA) (the numbers following the letter X denote
the number of amino acids at that position; X can be
any amino acid but X within a Y-X2-L preferably
represents the amino acids present in the Y-X2-L

30   sequence of the cytoplasmic domain of FcγRIIA or the γ

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chain of FcγRIII). Accordingly, it can be expected
that phagocytosis can be increased by multiplying the
number of copies of the core sequence, for example, in
FcγRIIA or in the γ chain of FcγRIIIA, or by

5   multiplying the number of copies of the second Y-X2-L
present in those core sequences. The specific amino
acids in this second Y-X2-L are important for
phagocytosis and appear to provide specificity to the
phagocytic signal. It is also expected that phagocytic

10   activity can be increased (as compared to the wild type
gamma chain) by, for example, inserting the FcγRIIA
second Y-X2-L into the γ chain of FcγRIIIA (as compared
to the wild type gamma chain). Furthermore, it is
expected that inserting the second cytoplasmic domain

15   Y-X2-L of the γ chain of FcγRIIIA (or both the first
and second cytoplasmic domain Y-X2-L of the γ chain)
into the ζ chain of FcγRIIIA will increase the
phagocytic activity of the ζ chain. Further, the
inclusion of two additional Y-X2-L or Y-X3-I motifs to

20   FcγRIIB (which itself is non-phagocytic) renders this
receptor phagocytic (this includes adding a variation
of the Y-X2-L, Y-X3-I, to the carboxyterminal portion
of the cytoplasmic domain). As indicated above,
fibroblasts and fibroblast-like cells (for example, COS

25   cells) can be used to assess the operability of a
particular receptor construct.
     The above-described chimeras of the invention can
be constructed by the polymerase chain reaction (PCR)
(Horton et al, Biotechniques 8:528 (1990)) using as

30   templates appropriate receptor cDNA and appropriate

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oligonucleotides. PCR products can be directly cloned
into an expression vector, for example, pSVL, and
confirmed by complete sequencing. The expression of
the chimeric receptors can be assayed by flow cytometry
5   using anti-Fcγ receptor mAbs and phagocytic function
can be evaluated following incubation of IgG-sensitized
RBCs.

More specifically, two step overlap extension PCR,
a technique that allows introduction of mutations into
10   any part of a PCR fragment, can be used to generate the
chimeric molecules of the invention, as well as the
mutated/ truncated receptors described herein. In the
first step in overlap extension PCR, two primer pairs,
1a and 1b and 2a and 2b, are used to generate two
15   overlapping fragments, 1 and 2. In step 2, when these
two fragments are mixed, denatured and reannealed, the
3' end of the sense strand of fragment 1 anneals to the
3' end of the antisense strand of fragment 2. This
overlap can be extended to form the entire recombinant
20   product and can be amplified by PCR using primers 1a
and 2b. The overlap region is determined by primers 1b
and 2a and can contain any sequence as long as parts of
the oligomers are complementary. This region is where
base changes are incorporated when the technique is
25   used for site directed mutagenesis. Alternatively, the
overlap can be designed to make a clean joint between
two fragments from two different DNA molecules to form
a chimeric molecule. For construction of chimeric
mutants, primers 1b and 2a are designed to contain
30   regions from both contributing molecules so that

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fragments 1 and 2 can anneal. For example, to
construct the chimera containing the FcγRIIIAα
extracellular region and the transmembrane and
cytoplasmic domains of the γ chain, the following 2

5   pairs of oligomer primers are used (primer 1b is shown
3'-5'):

        1a.5'ACGATGTCTAGAGGTGACTTGTCCACTCC3' (sense)
        1b.3'GGTGGACCCATGGTTGAGACGATATAGGAC5' (antisense)
        2a.5'CCACCTGGGTACCAACTCTGCTATATCCTG3' (sense)
10   2b.5'ATGGCGAGCTCTCCGGTAAACAGCATCTGAG3' (antisense)

        Xba1 and Sac1 restriction sites can be introduced
in primers 1a and 2b respectively so that the final PCR
product encoding the chimeric receptor can be ligated
in the proper orientation into, for example, an SV40

15   based expression vector (e.g., PSVL) restricted with
Xba1 and Sac1. To produce truncated molecules, stop
codons can be introduced via primers 1b and 2a. In a
similar fashion, tyrosine codons can be replaced by
phenylalanine codons and serine or threonine codons by

20   alanine codons.
        Target cells and modes of administration:
        As noted above, the present invention can be used
to treat patients that are predisposed to an increased
risk of infection. Such patients include, but are not

25   limited to, those suffering from liver disease
resulting, for example, from alcoholic cirrhosis, from
kidney disorders, such as end-stage renal disease, and
from pulmonary disorders including cystic fibrosis and

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ARDS. AIDS patients are also appropriate candidates
for treatment in accordance with the present invention.
In each instance, treatment is effected by increasing
the phagocytic potential of cells of the patient.

5   In the case of pulmonary disorders, the receptor-
encoding sequence can be administered to the cells of
the lung, including macrophages, in the form of an
aerosol. The encoding sequence can be present in the
aerosol as a particle (e.g. liposome or non-infectious

10   bacteria, for example, _Listeria_) that is phagocytosed
by the pulmonary macrophages. The encoding sequence
can also be present in a viral vector.

          Viral vectors can also be used to introduce the Fc
receptor-encoding sequence of the invention into cells

15   of the pulmonary tree, including fibroblasts,
epithelial cells and other cells present in the lung.
The vectors can be introduced as an aerosol and can
take the form of a replication defective herpes or
adenoviral vector. Retroviral vectors can also be

20   used, as well as other viral vectors. (See, generally,
Bajocchi et al, Nat. Genet. 3:229 (1993); Lemarchand et
al, Circ. Res., 72:1132 (1993); Ram et al, Cancer Res.
53:83 (1993); Crystal, Am. J. Med. 92:44s (1992);
Yoshimura et al, Nucl. Acids Res. 20:3233 (1992);

25   Morecki et al, Cancer Immunol. Immunother. 32:342
(1991); Culver et al, Hum. Gene Ther. 1:399 (1990);
Culver et al, Transplant. Proc., 23:170 (1991)).

          The Fc receptor-encoding sequences of the
invention can also be introduced into cells such as T

30   cells thereby rendering them phagocytic. The

25

advantages of phagocytic T cells are clear,
particularly in combating infections that accompany
diseases such as AIDS. The abundance of T cells is
such that by transforming them with the Fc receptor
5   encoding sequences of the invention, the phagocytic
capacity of the blood is substantially increased.

T cells can be rendered phagocytic by transforming
them *in vitro* with, for example, a viral vector
containing a sequence encoding an Fc receptor (e.g.
10   FcγRIIA). Techniques such as electroporation can also
be used. The transformed T cells can then be
reintroduced into the patient from which they were
derived. Example X details the transformation of T-
cells with FcγRIIA and the results presented
15   demonstrate that phagocytic activity is conferred on
these cells. In addition, FcγRIIA is phosphorylated in
the T-cells when activated, similar to the
phosphorylation observed in activated monocytes and
macrophages. FcγRIIA activation in these T-cells leads
20   to tyrosine kinase activation and phosphorylation. The
T-cell tyrosine kinase ZAP-70 is activated
(phosphorylated) upon FcγRIIA activation in T-cells. B
lymphocytes are less abundant than T lymphocytes, but
they too can be rendered phagocytic using similar
25   protocols (see Example VII).

Further, blood monocytes can be transformed *ex
vivo* with the receptor-encoding sequence of the
invention (using, for example, physical techniques such
as electroporation, or vectors, including viral vectors
30   (e.g., retroviral vectors, adenoviral vectors, or

herpes viral vectors); liposomes and <u>Listeria</u> can also
be expected to be useful in transforming monocytes and
then reintroduced into the patient). This protocol is
particularly advantageous when the liver or spleen is
5   the target site.

In addition to the above, the present invention
can be used with patients suffering from immune complex
diseases such as lupus erythematosus and rheumatoid
arthritis to increase local clearance of circulating
10   immune complexes so as to prevent their deposition in
tissues, such as the kidney, and in the joints. This
increase can be effected by stimulating liver and
splenic macrophage phagocytic potential using protocols
such as those described herein.

15   It will be appreciated from a reading of the
foregoing that, depending on the target cell and the
effect sought, various methods can be used to introduce
receptor-encoding sequence into the cell (in addition
to electroporation noted above, calcium phosphate as
20   well as other techniques can be used to introduce naked
DNA). It will also be appreciated that the gene
therapy approach to enhancing phagocytic potential can
be used alone or in combination with the drug therapy
approach described above. The combination therapy
25   makes it possible to increase the number of naturally
occurring receptors and at the same time effect the
selective expression of receptors of a particular
functional type.

The following non-limiting Examples describe
30   certain aspects of the invention in greater detail.

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EXAMPLE I

In Vivo Administration of hrM-CSF Increases
Splenic Macrophage Fcγ Receptors

Human recombinant macrophage colony stimulating
factor (hrM-CSF) was studied *in vivo* using an
established model in the guinea pig (Schreiber et al,
J. Clin. Invest. 51:575 (1972)). Adult male guinea
pigs were treated for 5 days with hrM-CSF (500 µg/kg)
and splenic macrophage FcγR function and protein
expression were assessed by i) the splenic macrophage
clearance of IgG sensitized
^{51}Cr-guinea pig RBC (EA), ii) the *in vitro* binding of
EA by isolated splenic macrophage, and iii) FACS
analysis using monoclonal antibodies with specificity
for the two guinea pig splenic macrophage Fcγ
receptors, FcγR1,2 and FcγR2. Treatment with hrM-CSF
enchanced the clearance of EA by 72 ±5%. In addition,
a greater proportion of isolated splenic macrophages
from hrM-CSF treated animals bound EA *in vitro*: 80±7%
vs 48±4%(sham), p<0.001. *In vivo* hrM-CSF increased the
expression of both splenic macrophages Fcγ receptors:
81±6% and 130±10% for FcγR1,2 and FcγR2, respectively.
The lowest effective dose of hrM-CSF was 250 µg/kg,
increasing the expression of FcγR1,2 by 26±3% and FcγR2
by 42±4%. At this dose, the clearance of EA was also
enhanced. The effect of hrM-CSF required at least 4
days of treatment.

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EXAMPLE II

FcγIIA Mediates Phagocytosis and Receptor
Phosphorylation in a Fibroblast Cell Line

Experimental Protocols:

5   *Cell culture and reagents:*
           The SAR6 cell line was derived from primary
embryonic mouse fibroblasts in which both Src alleles
had been disrupted by homologous recombination using
the neomycin resistance gene (Thomas et al, Science
10   254:568 (1991)). D58 was derived from primary
embryonic mouse fibroblasts that were wild type for
Src. Cells were maintained in DMEM containing glucose
(4.5 mg/ml), glutamine (25 mg/ml), penicillin (100
U/ml), streptomycin (100 μg/ml) and 10% heat
15   inactivated fetal calf serum.

           Retroviral infections:
           FcγRIIA was inserted into the HindIII site of the
retroviral vector pLCX (Miller and Rosman,
Biotechniques 9:908 (1989)) under control fo the CMV
20   promoter. The resulting construct, pLNCX2A, was
transfected into the ecotropic packaging cell line,
Psi2. Two days after transfection, the cells were
diluted 1:20 and G418 resistant colonies were isolated
and assessed for virus production. The stock gave 1 x
25   10 G418 resistant colonies per milliliter. 0.1 ml of
viral stock was used to infect DS8 and SAR6 cells (2.5
x 10 cells per infection). Twenty four hours after

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infection, the cells were diluted 1:3 and allowed to
reach 80-90% confluence before assaying for cell
surface expression of FcγRIIA and for phagocytosis.
Transient infections were carried out due to the fact
5   that the G418 resistant phenotype of the SAR6 cell line
prohibited the selection of stable lines using this
retroviral vector.

        Flow cytometry:
        To determine the extent of FcγRIIA expression on
10   the cell surface of infected D58 and SAR6 cells,
samples were stained with fluorescein-labeled anti-
FcγRII mAb (IV.3) or with an isotype control (Indik et
al, J. Clin. Invest. 88:1766 (1991)). Fluorescence was
measured on a FACStar (Becton-Dickinson, Mountainview,
15   CA). 10,000 events were analysed in each case and mean
fluorescence intensities were estimated and contour
maps were generated using Consort 30 software.

        *Binding and phagocytosis of IgG-sensitized sheep
red blood cells (EA):*
20   EA was prepared as described previously (Indik et
al, J. Clin. Invest. 88:1766 (1991)), overlaid onto the
infected cells and incubated at 37°C for 30 minutes.
Unbound EA was removed by washing with PBS and the
plates stained with Wright-Geimsa to assess rosetting.
25   To determine phagocytosis, extracellular bound EA was
removed by exposure to hypotonic buffer before staining
with Wright-Geimsa.

30

Biotinylation of cell membranes:

Twenty four hours after infection, FcγRIIA-
infected and sham-infected SAR6 and D58 cells were
plated on 100 mm petri dishes. After a further twenty
5   four hours, the cells (2 x 10) were washed once with
PBS, overlaid with 1.0 ml of PBS containing 100 μl of 1
M NaHCO and 100 ml of 1 mg/ml biotin (Pierce, Rockford,
IL) and incubated at room temperature for 60 minutes.
One hundred μl of NHC1 was added and incubation
10   continued for a further 10 minutes. The cells were
washed once with PBS and lysed with 1.0 ml RIPA buffer
(1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 158
mM NaCl, 10-mM Tris pH7.2, 5 mM NaEGTA, 1 mM
phenylmethylsulphonyl fluoride, 1 mM NaVO) at 4°C for
15   30 minutes. FcγRIIA was immunoprecipitated from the
biotinylated cell membrane extract with anti FcγRII mAb
(Eisman and Bolen, Nature 355:78 (1992)) and analyzed
on a 7.5% SDS-polyacrylamide gel (PAGE). Immunoblots
were probed with avidin-horseradish peroxidase (BioRad,
20   1:1000 dilution), followed by Enhanced
Chemiluminescence reagents (Amersham Corp.) and
visualized using Kodak XAR-5 film.

Phosphotyrosine immunoblots:

FcγRIIA-infected and sham-infected D58 and SAR6
25   cells (2 x 10 cells per 100 mm petri dish) were
overlaid with 500 μl EA and incubated at 37°C for 30
minutes to activate FcγRIIA. After washing with PBS to
remove unbound EA, the bound EA was removed by exposure
to hypotonic buffer. Cells were lysed on the plates

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with 1.0 ml RIPA buffer at 40°C for 30 minutes and
phosphotyrosine containing proteins were
immunoprecipitated from the cell lysates using
polyclonal rabbit antisera UP28 (Huang et al, J. Biol.
5   Chem. 267:5467 (1992)). The immunoprecipitates were
analyzed on a 7.5% SDS-PAGE and immunoblots probed with
antiphosphotyrosine mAb, 4G10 (Huang et al, J. Biol.
Chem. 267:5467 (1992)).

In vitro immune complex kinase assay of Src-family
10 *protein tyrosine kinases from FcγRIIA infected Src- and*
Src+ cells:
FcγRIIA-infected and sham-infected SAR6 and D58
cells (2 x 10 cells per 100 mm petri dish), were lysed
with 1.0 ml RIPA buffer at 4°C, for 30 minutes.
15   Immunoprecipitations were performed by mixing cell
lysates with the following mAbs singly or in
combination: anti-Src (Lipsiche et al, J. Virol.
48:352 (1983)), anti-FcγRII (Rosenfeld et al, J. Clin.
Invest. 76:2317 (1985)), anti-Fyn (Huang et al, J.
20   Biol. Chem. 267:5467 (1992)) and rabbit anti-mouse
(RAM) gG. The immune complexes were incubated with
[γ^{32}P]ATP to allow autophosphorylation of the kinases
and phosphorylation of the substrate and were separated
by SDS-PAGE. The gel was washed with 1 N KOH at 55°C
25   for two hours to remove serine/threonine
phosphorylation (tyrosine phosphorylation is relatively
resistant to alkali) before exposure to Kodak XAR-5
film.

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Results of Phagocytosis and Phosphorylation Studies:

 Forty eight hours after infection of cell lines
D58 and SAR with a retroviral vector containing a
FcγIIA encoding sequence, cell surface biotinylation
5   followed by immunoprecipitation with anti-FcγRII mAb
demonstrated that the 40 kD receptor was present in the
membrane of both Src+ and Src- cells (Figure 1a).
Fluorescence histograms of FcγRIIA infected SAR6 and
D58 cells are shown in Figure 2. In this
10   representative experiment, sixty five percent of cells
expressed the receptor in SAR6 and eighty one percent
in D58 with mean fluorescence intensities of ninety
five and one hundred and fifty one, respectively. Both
Src- and Src+ cells incubated with IgG sensitized cells
15   (EA) bound and phagocytosed these immune complexes.
Forty three percent of cells phagocytosed EA in the
Src- mutant and seventy percent in D58. In contrast,
no binding or phagocytosis was observed in sham
infected cells.
20   To determine if the activated receptor was
phosphorylated in the Src- cell line, phosphotyrosine
containing proteins were immunoprecipitated from
activated and unactivated SAR6 and D58 infected cells.
Crosslinking of FcγRIIA with EA resulted in tyrosine
25   phosphorylation of the 40 kD FcγRIIA receptor protein
in both Src+ and Src- cells (Figure 1b).
 Although Src is not responsible for
phosphorylating FcγRIIA in SAR6 cells, FcγRIIA in these
mouse fibroblasts was able to act as a substrate for

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Src related tyrosine kinases. An *in vitro* immune
complex kinase assay was performed on lysates from SAR6
and D58 cells that had been infected with FcγRIIA.
Lysates were co-immunoprecipitated with antibodies
5   specific for the receptor protein and with antibodies
specific for either Src or Fyn kinases (Figure 3). The
co-immunoprecipitates were incubated with [γ^{32}P] ATP to
allow autophosphorylation of the kinase and
phosphorylation of FcγRIIA. FcγRIIA was phosphorylated
10   by Src in this *in vitro* assay (Figure 3, lane 5). Fyn
could also phosphorylate FcγRIIA, although to a lesser
extent when compared to Src (lane 6). In the absence
of the kinases, no phosphorylation of FcγRIIA was
observed (lanes 4 and 10) consistent with the lack of
15   tyrosine kinase sequences in the receptor. In the Src-
lysates, co-immunoprecipitation with Src and FcγRIIA
did not result in phosphorylation of the receptor (lane
11), but a low level of phosphorylation of FcγRIIA was
observed in co-immunoprecipitates of FcγRIIA and Fyn
20   (lane 12). This may reflect the efficiency of
phosphorylation of the receptor by Fyn; alternatively
the fibroblasts may express different amounts of the
two kinases.

EXAMPLE III

25   High Affinity Fcγ Receptor (CD64) Induces
        Phagocytosis in the Absence of its Cytoplasmic Domain

        Wild type (WT) and a mutant (MT) FcγRI, engineered
to omit the cytoplasmic domain (CYT), were transfected

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into COS cells and murine macrophages (P388D1). The
phagocytic potential of the transformed cells was
assessed using IgG-coated RBCs (EA) and RBCs conjugated
with Fab anti-human FcγR1 mAb (E-mAb). FcγR1, in

5   contrast to FcγRII, did not induce phagocytosis in COS
cells (assessed by electron microscopy) but did induce
a Ca^{2+} signal which required its CYT. However, both WT
and MT FcγRI induced phagocytosis in P388D1.
Phagocytosis by WT FcγRI was inhibited by the tyrosine

10   kinase inhibitor tyrphostin 23. Furthermore,
activation of FcγRI on monocytes with Fab anti-FcγRI
induced tyrosine phosphorylation of FcγRII, determined
by anti-phosphotyrosine immunoblots. FcγR1 thus
mediates a Ca^{2+} signal through its cytoplasmic domain

15   but not phagocytosis. FcγRI induced phagocytosis
therefore requires elements, present in macrophages but
absent in COS cells, that permit transmembrane
communication.

<u>EXAMPLE IV</u>

20   Structural Requirements of the Human Fc
Receptor FcγRIIA in Phagocytosis

The structural requirements of FcγRIIA in
phagocytosis were examined using COS-1 cells, which
lack endogenous Fc receptors, as the recipient in

25   transfection studies. FcγRIIA has two (Y282 and Y298)
tyrosine-containing core sequences, Y-X2-L, within a
cytoplasmic motif similar to that in other Ig gene
family receptors. Truncation of the cytoplasmic domain

at amino acid 268 or 280, to produce mutants lacking
both these tyrosines and both core sequences,
eliminated phagocytic activity even though these
transfectants bound IgG-sensitized cells efficiently.
5   Truncation at amino acid 303, deleting only the
terminal 8 amino acid and preserving both core
sequences, did not decrease phagocytosis. Substitution
of Y282 with phenylalanine (F) inhibited phagocytosis
and substitution of Y298 with F partially diminished
10   the phagocytic signal. Substitution with F of the
third cytoplasmic tyrosine (Y275) outside the conserved
motif did not alter phagocytosis. Replacement of Y282
or Y298 with lysine reduced phagocytosis further, but
replacing Y275 with lysine had little effect.
15   Replacement by F of either Y275 or Y298 in combination
with Y282 completely eliminated phagocytic function,
suggesting that they interact with Y282 in transmission
of the signal. In contrast, some phagocytic activity
was preserved in mutants containing Y282, but with F at
20   Y275 and Y298. Deletion of T284-L285 within the
Y282MTL core sequence also diminished phagocytosis.
The two core Y282-X2-L and Y298-X2-L sequences contain
an intervening stretch of amino acids with 2 prolines
suggesting an intervening non-helical structure. A
25   mutant, Δ287-291, in which 5 amino acids including the
2 prolines were deleted reduced phagocytic function.
The initial core cytoplasmic sequence Y282MTL and the
proline containing region between Y282 and Y298 are
important for transmission of the phagocytic signal by
30   FcγRIIA.

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EXAMPLE V

The Structure of the γ chain Fc Receptor
Subunit Determines Phagocytic Function of
Macrophage FcγRIII (FcγRIIIA)

5   A FcγRIIIA encoding sequence was transfected into
COS-1 cells to study its phagocytic function,
determined by electron microscopy, in the absence of
other Fc receptors. Co-transfectants of FcγRIIIA-α
with either γ or ζ gave equivalent cell surface
10   expression and binding of IgG-coated cells (EA), but γ
was 6 fold more effective than ζ in phagocytosis. To
delineate the region of the γ chain important in
phagocytosis, two deletion mutants, were constructed,
deleting the C-terminal 7 amino acids or deleting the
15   C-terminal 22 amino acids which have a tyrosine
containing conserved motif, Y-X2-L-X7-Y-X2-L, present
in several Ig gene super family receptors. The C-
terminal 7 amino acid deletion demonstrated minimally
reduced phagocytic activity, whereas the more extensive
20   deletion completely eliminated phagocytosis, suggesting
the importance of the conserved cytoplasmic motif. The
role of the conserved cytoplasmic tyrosines was then
examined. Conservative substitution by phenylalnine of
either of the 2 cytoplasmic tyrosines in the γ chain
25   significantly decreased Ca^{2+} signaling and reduced
phagocytosis by >99%. Tyrophostin 23 which alters
tyrosine kinase activity reversibly inhibited
phagocytosis, indicating that phosphorylation of γ
and/or downstream protein tyrosine kinase(s) is

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required for a phagocytic signal. Further, single
chain Fcγ receptor chimeras, consisting of the γ
cytoplasmic domain and the α extracellular domain with
the transmembrane domain of either FcγRIIIA-γ or FcγRI

5   were able to mediate a phagocytic signal. However,
single chain chimeras were not sufficient for full
phagocytic activity.

EXAMPLE VI

Examination of Phagocytosis by Chimeric Fcγ

10   Receptors

FcγRIIA avidly binds and phagocytoses IgG-
sensitized cells (EA), as assessed by electron
microsopy using the COS cell transfection model system,
but FcγRI and two other FcγRII isoforms, FcγRIIB1 and

15   FcγRIIB2, do not transmit a phagocytic signal although
they also bind EA avidly. Chimeric receptors of FcγRI
and FcγRII were constructed in order to further assess
the function of their transmembrane and cytoplasmic
domains in phagocytosis. Chimeric transfectants

20   consisting of the extracellular (EC) and transmembrane
(TM) regions of FcγRIIB2 and the cytoplasmic domain
(CYT) of FcγRIIA and chimeric transfectants consisting
of the EC of FcγRI and the TM and CYT of FcγRIIA were
efficient in phagocytosis. In contrast, phagocytosis

25   was greatly diminished by chimeras consisting of the EC
and TM of FcγRI and the CYT of FcγRIIA. In addition, a
chimeric transfectant bearing the EC from FcγRI, the TM

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from FcγRIIA and the CYT from FcγRI did not phagocytose
EA. These studies indicate that in this system: i) the
transmembrane domain of FcγRIIB2 is able to provide the
necessary structure to permit a phagocytic signal by

5   the cytoplasmic domain of FcγRIIA, ii) the
transmembrane domain of FcγRI is unable to transmit a
phagocytic signal to the cytoplasmic domain of FcγRIIA,
and iii) the transmembrane domain of FcγRIIA is unable
to confer phagocytic competence to FcγRI.

10   **EXAMPLE VII**

B-Cell Antigen Receptor Subunit Ig-γ
Mediates Phagocytic Signal

The B-cell receptor complex is composed of an
antigen recognition subunit noncovalently associated

15   with a membrane subunit consisting of heterodimers of
two chains, Ig-α and Igβ/γ, which are products of the
mb-1 and B29 genes. Both membrane Ig subunits contain
within their cytoplasmic regions a conserved sequence
implicated in intracellular signalling. Using COS cell

20   transfectants, the Fc receptor FcγRIIA, which is not
present in B-cells, has been shown to mediate a
phagocytic signal and to contain within its cytoplasmic
domain a sequence similar in some aspects to that of
Ig-α. Therefore, a FcγRIIA and Ig-α chimera was

25   constructed, consisting of the extracellular and
transmembrane domains of FcγRIIA and the cytoplasmic
domain of Ig-α. This chimeric receptor was expressed
in COS-1 cell transfectants, determined by flow

cytometry, and bound IgG-sensitized RBCs (EA)
efficiently. Furthermore, transfection of this
chimeric receptor into COS-1 cells conferred phagocytic
competence to COS-1 cells similar in extent to
5   transfection of the receptor FcγRIIA.

                            EXAMPLE VIII

               Alterations in Monocyte/Macrophage Fcγ
           Receptor Expression in the Acute Respiratory
                     Distress Syndrome (ARDS)

10   Monocytes from patients with ARDS were used to
examine potential alterations in Fcγ receptor
expression. Since macrophages may express all 3
classes of Fcγ receptors, specific mAbs for each class
of Fcγ receptor and flow cytometry were used to
15   quantitate Fcγ receptor expression. Patients with ARDS
met the following four criteria: i) acute bilateral
alveolar-type infiltrates on chest radiograph, ii)
severe hypoxemic respiratory failure with PaO/FiO </=
150 without PEEP, iii) absence of congestive heart
20   failure, and iv) having a presumed pre-disposing cause
of ARDS. Seven patients with ARDS were compared to 5
normal controls. Whether measured as percent of cells
expressing the Fcγ receptor or the difference in mean
fluorescence intensity (MFI), FcγRI was reduced in
25   patients with ARDS (ARDS=36.0 ± 6.3% [mean $\pm$ SEM] or
22.6 ± 7.0 MFI; normal = 52.8 ± 11.3% or 35.6 ± 6.4 MFI) and
FcγRIII was increased (ARDS = 15.6 ± 7.9% or 12.1 ± 4.9
MFI; normals = 0.8 ± 0.6% or 1.4 ± 1.2(MFI). No

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correlation was observed between decreased FcγRI and
increased FcγRIII expression, suggesting differential
regulation of these receptors *in vivo*. No significant
change was observed in the expression of FcγRII. Four
5   of seven patients with ARDS died. One patient was
restudied following recovery and Fcγ receptors returned
to normal values.

EXAMPLE IX

Fc Receptor Defect in Patients with Liver Disease

10   Experimental Protocols:

Patients:
Forty nine patients (16 women and 33 men) whose
mean (±SD) age was 55.2±8.3 years were studied. All
patients had biopsy proven alcoholic cirrhosis of the
15   liver and were followed up to a minimum period of two
years after study: six died within this period. Ten
alcoholic non-cirrhotic subjects (4 women and 6 men;
age 45±7 years) and, 20 healthy volunteers (6 women and
14 men; age 52±12 years) served as concurrent controls.
20   Patients were classified in three groups according to
their degree of liver insufficiency as assessed by the
Orrego index (Orrego et al, Gastroenterology 76:105
(1979)).

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Study Protocol:

Blood was drawn on admission for the following
measurements: (1) blood glucose and urea nitrogen,
sodium, potassium, chloride, total calcium, phosphate,
5   magnesium, creatinine, uric acid, total cholesterol,
triglycerides, LDL-cholesterol, HDL-cholesterol, serum
aspartate and alanine aminotransferases, gamma-glutamyl
transpeptidase, 5'-nucleotidase, alkaline phosphatase,
serum protein electrophoresis, complete blood count,
10   prothrombin time, activated partial thromboplastin
time, fibrinogen and alpha-fetoprotein; (2) serum lgG,
lgA and lgM, determined by radial immunodiffusion
(Behring Diagnostics, Madrid); (3) serum C4, determined
by hemolytic titration (Gaither et al, J. Immunol.
15   113:574 (1974)), and serum C3 and C3a desArg,
determined by radial immunodiffusion (Behring
Diagnostics); (4) plasma levels of zinc, measured by
absorption spectrophotometry (pye Unicam SP 190); (5)
circulating immune complexes, determined by [^{12}I]C1q
20   binding (Zubler and Lamber In: Bloom and David, eds.
In vitro Methods in Cell-Mediated and Tumor Immunity,
New York: Academic Press pp 565-72(1976)); (6)
peripheral-smear examination after Wright-Giemsa
staining to assess the presence of Howell-Jolly bodies
25   as an index of splenic function (Boyko et al, Am. J.
Clin. Pathol. 77:745 (1982)) (negative in all
patients); (7) macrophage Fcγ-receptor-dependent
clearance *in vivo*; and (8) Fcγ-receptor-mediated
recognition of sensitized cells by peripheral-blood
30   monocytes *in vitro*; and (9) abdominal ultrasound to

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assess for the presence of splenomegaly, which was
detected in 17 out of the 49 patients.

Preparation of human IgG anti-Rh(D):

Human IgG anti-RH(D) was prepared from serum from
5   a single donor (was HIV-1 negative by ELISA-Pasteur
Institute, Madrid-, Western Blott-Pasteur Institute,
Madrid- and the quantitative end-point dilution method)
by ammonium sulfate preciptation followed by Sephacryl
S-300 gel filtration and QAE ion-exchange
10   chromatography (Pharmacia, Madrid). No IgM was
detected by double immunodiffusion (Ouchterlony
analysis). The final IgG fraction was passed through a
Millipore filter and tested for pyrogenicity and
sterility. The final IgG fraction was HIV-1 negative
15   by ELISA (Pasteur Institute, Madrid), Western Blott
(Pasteur Institute, Madrid) and the quantitative end-
point dilution method (Ho et al, N. Engl. J. Med.
321:1621 (1989)).

Macrophage Fcγ-receptor-mediated clearance:
20   Clearance studies were performed as previously
described (Ruiz et al, N. Eng. J. Med. 322:717 (1990);
Frank et al, N. Engl. J. Med. 300:518 (1979); Schreiber
and Frank, J. Clin. Invest. 51:575 (1972)). In brief,
erythrocytes (RhD) were isolated from all subjects,
25   washed three times in physiologic saline,
spectrophotometrically standardized to a concentration
of 6.6x10 cells per milliliter, and radiolabeled with
^{51}Cr (potassium dichromate, Amersham, Buckinghamshire,

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England). An aliquot of cells was sensitized by adding to it drop by drop an appropriate dilution of the purified human IgG anti-Rh(D). The mixture was incubated at 37°C for 30 minutes, and the sensitized

5   ^{51}Cr-labeled erythrocytes were washed four times in saline and resuspended to a concentration of 3.3x10 per milliliter in Hanks' balanced salt solution (M.A. Bioproducts, Madrid). An aliquot of cells (usually 10 ml, with 2.5 μCi of radioactivity) was injected through

10   an antecubital vein, and the survival of red cells was determined in serial blood samples obtained over a period of 48 hours. Clearance curves were plotted by expressing the number of counts per minute at each time point as a percentage of the number of counts at 10

15   minutes, the zero point. The time required for clearance of the 50 percent of the inoculated IgG-coated red cells (half-time) was calculated and then correlated with clinical and serologic data. In addition, for the clearance on each day, the percentage

20   for the inhibition of clearance above control was calculated at 1, 1.5, 2, 8, 24 and 48 hours, according to the formula

$$\% \text{ inhibition} = 100 \times 1 - \frac{(CPMb - CPMx)}{(CPMb - CPMc)},$$

25   where CPMb denotes the number of counts per minute in a control subject who received an injection of unsensitized autologous red cells, CPMx the number of counts in a patient who received IgG-coated (sensitized) autologous red cells, and CPMc the number

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of counts in a control subject who received autologous
IgG-sensitized red cells. By means of this formula,
patients could be compared with controls studied on the
same day, and results could be expressed as the
5   percentage of change in clearance, where 100 percent
inhibition of clearance indicated that clearance in a
patient who received IgG-coated red cells (CPMx) was
identical to clearance in a control who received
unsensitized red cells (CPMb) (Friedman, J. Clin.
10   Invest. 75:162 (1985)). In three additional control
groups - five patients with alcoholic cirrhosis of the
liver, five non-cirrhotic alcoholic subjects, and five
healthy volunteers - the clearance of autologous ^{51}Cr-
labeled but unsensitized red cells and the clearance of
15   ^{51}Cr-labeled heat-damaged autologous red cells were
examined.

         Duplicate studes were performed in nine of the
patients with alcoholic cirrhosis of the liver in whom
severe infection had developed, six of the patients
20   with alcoholic cirrhosis of the liver without a history
of complications due to infection, and six controls.
The results of the repeat studies of clearance were
unchanged from those of the original studies in each
subject. Serum C3, C3a desArg, and C4 were measured to
25   assess complement activation during the clearance of
IgG-coated red cells. No significant complement
activation was observed in any of the patients included
in the present study.

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Number of IgG (Anti-RhD) molecules per red cell:

The number of IgG molecules per red cell was
determined as previously described with the use of
^{12}I-labeled anti-IgG reagent (Cines and Schreiber, N.

5   Engl. J. Med. 300:106 (1979)). Clearance studies were
always performed with erythrocytes sensitized so that
approximately 600 molecules of IgG were present on each
red cell. When Fcγ-receptor-dependent recognition by
blood monocytes was studied *in vitro*, each red cell

10   (RhD) was coated with 400, 800, or 1600 molecules of
IgG.

Binding of IgG(Anti-RhD)-coated red cells:

The recognition of IgG-coated red cells (RhD) by
monocytes isolated was determined as previously

15   described (Gomez et al, J. Reticuloendothel. Soc. 31:24
(1982); Schreiber et al, J. Clin. Invest. 56:1189
(1975)). In brief, confluent monolayers of 5.5 x 10
monocytes were obtained from defibrinated blood after
density-gradient centrifugation (Ficoll-Isopaque) and

20   plastic adherence to petri dishes (Nunc, Amsterdam).
An aliquot of 2 x 10^7 ^{51}Cr-labeled, IgG-coated red cells
(RhD) was added to each monocyte monolayer. The petri
dishes were then incubated at 37°C in an atmosphere of
5 percent carbon dioxide for 45 minutes, washed to

25   detach unbound red cells, and treated with 0.086 M EDTA
solution to remove adherent monocytes and monocyte-
bound IgG (Anti-RhD)- sensitized red cells. The
treatment with EDTA removed all adherent monocytes and
all radioactivity. The percentage of

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^{51}Cr-labeled and IgG-sensitied red cells (RhD)
recognized by peripheral-blood monocytes was determined
according to the formula:

$$\%\text{red-cell IgG bound to monocyte monolayers} = \frac{\text{cpm for IgG (anti-RhD)-coated red cells removed with EDTA}}{\text{cpm for IgG (antiRhD)-coated red cells added to monocyte monolayers}} \times 100.$$

No phagocytosis of anti-RhD-sensitized erythrocytes by
5   peripheral blood monocytes occurs under the
experimental conditions (Gomez et al, J.
Reticuloendothel. Soc. 31:241 (1982); Schreiber et al,
J. Clin. Invest. 56:1189 (1975)). The studies were
repeated in 6 controls, 6 non-cirrhotic alcoholic
10   patients, 9 of the patients in whom severe infection
developed and 6 of the patients with no history of
infectious complications; the results of the repeat
studies were unchanged from those of the original
studies in each subject.

15   *Preparation of IgG2b-sensitized red cells:*
      Antibody-sensitized sheep erythrocytes (EA) were
prepared as previously described (Rossman et al, Exper.
Hematol. 21:177 (1993)). In brief, 1 x 10 sheep red
blood cells in 1.0 ml of 0.01 mol/L EDTA buffer were

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sensitized by adding mouse monoclonal antibody Sp2/HL,
subclass IgG2b (Serotec Ltd., Bicester, Oxon), in 0.1
ml at 37°C for 1 hour. The final antibody dilutions
used to prepare these cells were between 1:10 and 1:80.

5   The IgG-sensitized (coated) sheep red cells were washed
twice and resuspended in HBSS to a final concentration
of 1 x 10 cells/ml. In addition, a polyclonal 7S IgG
rabbit anti-sheep red blood cell (Cordis Laboratories)
was also used to prepare polyclonal IgG-coated red

10   blood cells. The final antibody dilution used to
prepare these cells was 1:1000.

       *Monocyte recognition of sheep IgG-sensitized red
       blood cells*:
              Monocyte *in vitro* recognition of IgG-sensitized

15   red cells was assessed as previously reported (Rossman
       et al, Exper. Hematol. 21:177 (1993); Schreiber et al,
       N. Engl. J. Med. 316:503 (1987)). In brief, 1 x 10
       IgG-coated red cells or control unsensitized red cells
       were added to monocyte monolayers containing 1 x 10

20   cells. These cells were incubated at 4°C or 37°C in
       phosphate buffer at an ionic strength of $\mu = 0.07$ or μ
       = 0.15, respectively. After 2 hours, the plates were
       washed and stained with Wright's Giemsa. Two hundred
       (200) monocytes were counted under light microscopy in

25   a blinded fashion to assess the number of IgG-
       sensitized red blood cells bound per monocyte.
       Monocytes binding >3 red blood cells/monocyte were
       determined. These experiments were performed in 5
       patients of each alcoholic cirrhosis of the liver

30   groups (I, II and, III), 5 alcoholic non-cirrhotic

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subjects and 5 normal volunteers. The experiments were
repeated in these same patients and controls at least
one year after the initial studies. No significant
variations were found between the initial experiments
5   and the ones performed after more than one year.

      HLA typing:
      HLA typing was performed by the tissue-typing
laboratory of the Virgen del Rocio University Hospital,
Seville, Spain.

10   *Assessment of nutritional status*:
      Nutritional status was evaluated according to
anthropometric, biochemical, and immunologic
measurements (Blumonkrantz et al, Am. J. Clin. Nutr.
33:1567 (1989); Harvey et al, Am. J. Clin. Nutr.
15   33:1587 (1989); Feliffe, Wo 1966, No. 53, Geneva,
Switzerland; Bristian et al, JAMA 235:1567 (1976)).
Dry body weight, relative body weight, and the percent
ideal body weight were also determined. The
anthropometric data were compared with standard values
20   for the local population (Jaurrieta, Med. Clin. 81:584
(1983)). Serum albumin and transferrin were measured
to evaluate the serum protein level. Malnutrition was
classified according to previously established criteria
(Blumenkrantz et al, Am. J. Clin. Nutr. 33:1567 (1980);
25   Harvey et al, Am. J. Clin. Nutr. 33:1586 (1980);
Feliffe, WO 1966 No. 53, Geneva, Switzerland; Bristian
et al, JAMA 235:1567 (1976); Jaurrieta, Med. Clin.
81:584 (1983); O'Keefe et al, Lancet 2:615 (1980)) as

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marasmus, kwashiorkor, or mixed type. All malnourished
patients had m... nutrition of the mixed type. A high
incidence of protein-calorie malnutrition of the mixed
type was observed in 17 of the 49 patients (35

5   percent). Total body weight did not change. Cutaneous
hypersensitivity responses to standard concentrations
of four antigens-purified protein derivative,
Trycophyton rubrum, Candida albicans, and
streptokinase-streptodornase- were used to evaluate

10   cell-mediated immunity as previously described (Harvey
et al, Am. J. Clin. Nutr. 33:1586 (1980); Blackburn et
al, J. Parenter. Enteral. Nutr. 1:11 (1977)). A
response was considered positive when the diameter of
induration was more than 5 mm. A normal response was

15   indicated by a positive response to either three or
four antigens, an abnormally low response by a positive
response to either one or two antigens, and anergy by a
lack of positive response to any of the four antigens.

     Statistical analyses:

20   The *in vivo* clearance curves were analyzed at the
time points to calculate a P value for the difference
between the controls and patients by Student's t-test.
The *in vitro* Fcγ-receptor-dependent recognition of red
cells by monocytes and the clearances in patients and

25   controls were assessed with the Wilcoxon rank-sum test
for unpaired data. The relation of the clearance rate
(as half-time) or monocyte Fcγ-receptor-dependent
recognition of IgG-coated red cells *in vitro* to the

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seologic tests was analyzed with the Spearman rank-correlation test.

Clearance Study Results:

Clearance studies were performed in the 49
5   patients with alcoholic cirrhosis of the liver
fulfilling the inclusion criteria of this study. The
results demonstrated that the clearance of IgG-coated
red cells was significantly impaired ($p < 0.001$) (Figure
4). At 1 and 1.5 hours, the mean ($\pm$SEM) inhibition of
10   macrophage Fcγ-receptor-mediated clearance was 47 $\pm$ 3
and, 53 $\pm$ 3 percent, respectively. Clearance was
inhibited by more than 15 percent in 37 patients and,
by 5 to 12 percent in 6. In contrast, the clearance of
unsensitized red cells and of heat-damaged red cells in
15   the patients did not differ from the clearance of these
cells in the non-cirrhotic alcoholics and healthy
volunteers (Figure 4).
Patients were classified in three groups according
to their degree of liver insufficiency as assessed by
20   the Orrego index. Clearance studies of those three
groups of patients are represented in Figure 5. The
results demonstrated that the clearance of IgG-coated
red cells was significantly impaired ($p < 0.001$) in
patients with moderate (Patients II or group II) and
25   severe (Patients III or group III) liver insufficiency.
At 1 and 1.5 hours, the mean ($\pm$ SEM) inhibition of
macrophage Fcγ-receptor-mediated clearance was 47 $\pm$ 3
percent and 66 $\pm$ 4 percent, respectively, for group II

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patients. At 1 and 1.5 hours the mean ($\pm$SEM)
inhibition of macrophage Fcγ-receptor-mediated
clearance of IgG-coated red cells was impaired in
patients with mild liver insufficiency (Patients I or

5   group I), (Figure 5), but the difference was not
significant.

   The patients were followed up for at least two
years after the clearance studies were initially
performed. Six patients died, two of massive

10   hemorrhage from ruptured esophgeal/gastric varices
(15th and 17th month of follow up, respectively), two
spontaneous bacterial peritonitis by *E. coli* (14th and
20th month of follow up, respectively), and two Gram-
negative sepsis due to *E. coli* and (16th and 21st month

15   of follow up, respectively). Eleven patients had
severe infection: five had spontaneous bacterial
peritonitis (*E. coli*) and, six had sepsis (due to *E.
coli* in three, *Staphyloccus aureus* in one, in one, and
Serratia marcescens in one). When the clearance of

20   IgG-coated red cells in the patients with severe
infection was compared with the clearance in the
patients without infection, those with infection were
found to have a significantly longer half-time
(126.2$\pm$22 vs. 32.2$\pm$18 hours; $p<0.001$)(Figure 6). The

25   clearance of IgG-coated red cells was analyzed in the
patients (half-time) in relation to various parameters
of liver impairment (SGOT, SGPT, GGT, 5'-nucleotidase,
bilirubin -total, direct and indirect-, P.T., aPTT,
fibrinogen and serum albumin). None of these

30   parameters, including the presence of splenomegaly,

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correlated with the extent of impairment of clearance
of IgG-coated red cells.

    Isolated peripheral blood monocytes were also
studied (Figure 7). Erythrocytes from a single Rh(D)-
5   positive donor were sensitized with three different
concentrations of IgG-antiRh(D) (400, 800, and 1600 IgG
molecules per red cell). Monocytes isolated from the
patients bound fewer IgG-coated red cells than did
those from the controls, but the difference was not
10   significant. There was no correlation between the
extent of binding by monocytes and the degree of
impairment of clearance of IgG-coated red cells. No
difference was observed between this alteration in
monocyte FcγRI in patients in whom severe infection
15   developed and those in whom it did not.

    The function of monocyte FcγRII was assessed *in
vitro* by the binding of IgG2b-coated red blood cells
(Figure 8). Peripheral blood monocytes isolated from
patients with cirrhosis of the liver bound less IgG2b-
20   sensitized red cells than monocytes from non-cirrhotic
alcoholic subjects or monocytes from normal volunteers,
but the difference was not significant.

    Seven patients had elevated levels of circulating
immune complexes. The clearance of IgG-coated red
25   cells in these patients did not differ from that
observed in the patients in general (Figure 9).
Furthermore, there was no correlation in these five
patients between the level of circulating immune
complexes and the extent of impairment of the
30   recognition of IgG-coated red cells by monocytes.

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Neither the clearance of IgG-sensitized
erythrocytes, nor the recognition *in vitro* of IgG-
coated red cells or IgG2b-coated red cells by monocytes
from the patients correlated with their sex, age, time
5   from diagnosis of alcoholic cirrhosis of the liver or
with any of the serologic measurements, including the
immunoglobulin level. Furthermore, there was no
relation between either the clearance of IgG-coated red
cells or their recognition *in vitro* by monocytes and
10   the HLA haplotype, or the nutritional status of the
population studied.

The plasma zinc level was 18.4 ± 0.7 μmol per
liter (120 μg per deciliter) in healthy volunteers and
12.7 ± 1.3 μmol per liter (83.3 ± 3.7 μg per deciliter)
15   in the patients with alcoholic cirrhosis of the liver
(p<0.001). However, there was no correlation between
the plasma zinc level and the degree of impairment of
clearance *in vivo* or the monocyte recognition of IgG-
coated red cells *in vitro*. Similarly, malnutrition was
20   not necessarily linked with greater impairment of the
clearance rate or a lower value for *in vitro* monocyte
recognition of IgG-sensitized red cells. The
prevalence of malnutrition was significantly higher in
the patients with either moderate or severe liver
25   insufficiency (groups II and II, respectively)
(p<0.001). However, neither the macrophage Fcγ-
receptor-mediated clearance nor the binding of IgG
(Anti-RhD)-coated red cells or the binding of IgG2b-
coated red cells by monocytes correlated with the

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nutritional status of these patients, as indicated by
anthropometric, biochemical, and immunologic values.

EXAMPLE X

T-Cells Transfected with FcγRIIA

5   Experimental Protocols:

        Cell lines and antibodies:
        The Jurkat T-cell line J32 and the CD2-CD28-CD3+
        variant J32-3.2 have been described previously (Makni
        et al, J. Immunol. 146:2522 (1991) and Sancho et al, J.
10   Immunol. 150:3230 (1993)). These cell lines were
        maintained in RPMI 1640 containing 10% heat inactivated
        FCS (Hyclone Laboratories, UT), 2mM L-glutamine,
        penicillin (100 U/ml) and streptomycin (100 U/ml). The
        following antibodies were used in this study: anti-CD2
15   mAbs 9.6 (Sancho et al, J. Immunol. 150:3230 (1993))and
        9.1 (Yang et al J. Immunol. 137:1097 (1986)), anti-CD3
        mAb 64.1 (Hansen et al, In Leukocyte Typing, Bernard et
        al eds. Springer-Verlag, New York p. 195 (1984)) and
        anti-FcγRII mAb IV.3 (Fanger et al, Immunol. Today
20   10:92 (1989)).

        *Construction of the FcγRIIA expression vector and
        DNA transfer into J32 and J32-3.2 cell lines:*
        FcγRIIA cDNA was isolated from the plasmid pKC4
        (Hibbs et al, Proc. Natl. Acad. Sci. USA 5:2240 (1988))
25   using EcoR1 and the fragment was blunt ended using

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Klenow polymerase. The FcγRIIA cDNA was then inserted
into the SmaI site of plasmid pGSE1731 (Greaves et al,
Cell 56:979 (1989)) under control of the human β-globin
gene promoter and enhancer sequences. pGSE1731

5   contains 4.9 Kb of the human β-globin gene including
1.5 Kb of sequences upstream of the CAP site and the
internal and 3' enhancer regions. This plasmid also
contains the CD2 3' .enhancer region which confers T-
cell specific, position-independent gene expression

10   (Greaves et al, Cell 56:979 (1989)). The resulting
plasmid, pGSE2A was introduced into the J32 and J32-3.2
cell lines by electroporation using methods previously
described in detail (Sancho et al J. Immunol. 150:3230
(1993)). Prior to electroporation, pGSE2A was

15   linearized by digestion with NotI. Each
electroporation was carried out using 30 μg of
linearized pGSE2A and 5 μg of pcEXV Neo linearized with
EcoRI. After electroporation, the cells were cultured
for seven days in the presence of 0.3 mg/ml G418 and

20   assayed for FcγRIIA expression by flow cytometry.
FcγRIIA expressing cells were enriched by
immunomagnetic positive selection using magnetic
particles coated with IgG (Dynal Inc., Fort Lee, NJ).
Cells were cultured in flat buttomed microtitre wells

25   (approx. 100 cells per well) and clones were selected
and analyzed for FcγRIIA expression by flow cytometry.

                              *   *

Tyrosine Phosphorylation Results:

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Stimulation of the T-cell receptor (TCR)/CD3
complex in Jurkat T-cells induces the tyrosine
phosphorylation of proteins including the TCR-
associated ζ chain, the ZAP70 tyrosine kinase and the
5   CD3ε complex (Weiss, Cell 79:209 (1993)). Similarly,
in the members of the IgG family of receptors,
induction of tyrosine phosphorylation accompanies
receptor activation (Samelson and Klausner, J. Biol.
Chem. 267:24913 (1992)) and, accordingly, studies were
10   conducted to determine if stimulation of FcγRIIA in the
T-cell transfectants J32/FcγRIIA and J32-3.2/FcγRIIA
induced tyrosine phosphorylation. The mutant J32-3.2
cell line is deficient in the induction of tyrosine
phosphorylation signalling pathways leading to impaired
15   induction of phosphorylated ZAP70, ζ chain and CD3ε
after TCR crosslinking (Sancho et al, J. Immunol.
150:3230 (1993)). The activation of the Src-related
tyrosine kinase (SRTKs) p56lck and p59fyn is also
defective in this mutant (Sancho et al, J. Immunol.
20   150:3230 (1993)).
Stimulation of FcγRIIA by crosslinking with anti-
FcγRII antibody followed by immunoprecipitation with
anti-phosphotyrosine antibody (Huange et al, J. Biol.
Chem. 267:5467 (1992)), showed that the 40 kD FcγRII
25   receptor is phosphorylated on tyrosines in both wild-
type J32 and in the mutant J32-3.2 transfectants
(Figure 10, lanes 4-11 (the position of the 40 kD
receptor is indicated with an arrow).

Phaqocytosis Results:

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FcγRIIA cDNA was expressed in the wild type Jurkat
T-cell line J32 and in the mutagenized J32 variant,
J32-3.2. As noted above, the J32-3.2 cell line is CD2-
CD28-CD3+ and exhibits reduced signal transduction
5   capabilities after TCR/CD3 stimulation, with respect to
tyrosine phosphorylation pathways and GTP binding
mechanism (Sancho et al, J. Immunol. 150:3230 (1993)).
Calcium mobilization and IL2 promoter activity induced
after TCR stimulation are also impaired (Sancho et al,
10   J. Immunol. 150:3230 (1993)). Fluorescence histograms
of J32/FcγRIIA and J32-3.2/FcγRIIA stable
transfectants, and FcγRIIA expressing clones isolated
from these transfected cells, are shown in Figure 11.
        The ability of these T-cell transfectants to
15   phagocytose IgG-sensitized cells was assessed by
incubation with IgG coated sheep erythrocytes (sEA).
In both the wild type J32 and mutant J32-3.2
transfectants, a number of the cells were able to
phagocytose the sEA (Figure 12). The results of
20   several experiments with (a) bulk cell stable FcγRIIA-
transfectants and (b) FcγRIIA clones are shown in Table
1. The data indicate that these T-cell transfectants
phagocytose EA and that phagocytosis by the J32-3.2
mutant transfectants was reduced compared to the wild
25   type cells.

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TABLE 1

Phacocytosis of Sheep EA by FcγRIIA bulk cell
stable transfectants of J32 and J32-3.2 cell lines.

		J32 / FcγRIIA			J32-3.2 / FcγRIIA	
5		%	P1		%	P1
	cP1					
	1.	17	28	-	-	-
	2.	33	22	5	6	7
	3.	25	40	13	18	21
10	4.	32	53	17	21	24
	5.	31	51	-	-	-
	6.	14	21	6	8	9

P1 is the phagocytic index, i.e., the number of
erythrocytes ingested per 100 cells. The corrected P1
15   value (eR1) is included in the J32-3.2 / FcγRIIA column
to take into account the lower MF1 value observed in
these transfected cells compared to the J32/FcγRIIA
transfected cells. % = % phagocytic cells.

Considering that 70%-100% of the cells are
20   expressing FcγRIIA in these transfectants, and
presumably are mediating phagocytosis through this
receptor, the levels of phagocytosis observed are
relatively low when compared, for example, to COS-1

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fibroblasts transfected with FcγRIIA (Indik et al, J.
Clin. Invest. 88:1766 (1991)). However, the ingestion
of the erythrocytes appears to be mediated via a
genuine phagocytic process as preincubation of the
5   cells in 10μg/ml cytochalasin-D, a compound which
inhibits actin polymerization (a process that is
necessary for phagocytosis) (Indik et al, J. Clin.
Invest. 88:1766 (1991)), abolished phagocytosis in
these cells. Also phagocytosis was inhibited when the
10 transfectants were incubated with sEA at 0°C instead of
37°C.

EXAMPLE XI

Induction of Phagocytosis by a Protein Tyrosine Kinase

Using the COS-1 cell experimental model to define
15 the structural requirements for phagocytosis, it has
been established that isoforms of each of the three
classes of the Fcγ receptors FcγRI, FcγRII and FcγRIII,
are able to transmit a phagocytic signal in transfected
COS-1 cells and that both FcγRI and FcγRIIIA require
20 the γ subunit for this signalling event. To determine
the *in vivo* kinase important for γ chain mediated
phagocytosis, the monocyte/macrophage protein tyrosine
kinase Syk, shown to be associated with the γ chain in
monocytes and macrophages, was co-transfected (Yagi et
25 al, Biochem. Biophys. Res. Commun. 200:28 (1994)). The
expression vectors used were as follows: Syk (full
length cDNA) - pME18S; FcγRI - pKC4; FcγRIIIA - pSVL;

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and γ - pSVL. Syk dramatically enhanced phagocytosis
mediated by both FcγRI/γ and FcγRIIIA/γ (FcγRI/γ,
8.0±2.0 fold; FcγRIIIA/γ, 6.1±0.6 fold) and, in
addition, increased the number of cells able to mediate

5   phagocytosis (FcγRI/γ, 3.6±0.5 fold; FcγRIIIA/γ,
3.0±0.2 fold). Two γ chain cytoplasmic YXXL sequences
were required but neither the cytoplasmic domain of
FcγRI nor FcγRIIIA was necessary for the Syk effect.
Syk expression also enhanced (5-7 fold) FcγRI and

10   FcγRIIIA phagocytosis mediated by the ξ chain, a
subunit homologous to γ, but did not increase the level
of phagocytosis to that observed for the γ chain. The
action of Syk was less pronounced (1.5±0.2 fold) for
the phagocytic FcγRII receptor, FcγRIIA, which does not

15   require the γ chain for phagocytosis. However, Syk
stimulated phagocytosis (6.0±1.0 fold) by the poorly
phagocytic FcγRII receptor FcγRIIB2, which contains
only a single YXXL sequence, when an additional
tyrosine containing sequence, YMTL, was introduced. No

20   enhancement of FcγRI/γ or FcγRIIIA/γ mediated
phagocytosis was observed when Fyn, a protein tyrosine
kinase of the Src family which is also expressed in
monocycte/macrophages, was co-transfected with FcγRI/γ
or FcγRIIIA/γ. Similarly, no enhancement was observed

25   when the protein tyrosine kinase ZAP-70, of the Syk
family of kinases, was used. These findings indicate
that there is specificity of Syk for γ chain sequences.

                        *   *   *   *

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All documents cited hereinabove are incorporated
in their entirety by reference.

W... le the invention has been described with
respec: to what is presently regarded as the most
5   practical embodiments thereof, it will be understood by
those of ordinary skill in the art that various
alterations and modifications may be made which
nevertheless remain within the scope of the invention
as defined by the claims which follow.

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WHAT IS CLAIMED IS:

1. A method of increasing the phagocytic
potential of cells present in a mammal comprising
introducing into said cells a DNA molecule coding for
an Fc receptor under conditions such that said DNA
molecule is expressed and said Fc receptor thereby
produced and the phagocytic potential of said cells
thereby increased.

2. A method according to claim 1 wherein said
cells are phagocytic prior to introduction of said DNA
molecule.

3. The method according to claim 1 wherein said
cells are macrophages.

4. The method according to claim 1 wherein said
cells are monocytes.

5. The method according to claim 1 wherein said
cells are non-phagocytic prior to introduction of said
DNA molecule.

6. The method according to claim 5 wherein said
cells are fibroblasts.

7. The method according to claim 5 wherein said
cells are T cells or B cells.

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8. The method according to claim 1 wherein said cells are lung cells.

9. The method according to claim 1 wherein said DNA molecule is present as an insert in a viral vector.

10. The method according to claim 1 wherein said DNA molecule is present in a liposome.

11. The method according to claim 1 wherein said DNA molecule is present in a bacterium.

12. The method according to claim 1 further comprising contacting said cells with an effective amount of a drug that increases the phagocytic potential of said cells.

13. The method according to claim 12 wherein said drug is γ-interferon, an estrogen or estrogen analog, M-CSF or GM-CSF.

14. A method of increasing the phagocytic potential of cells of a mammal comprising:
    i)   removing cells from said mammal,
    ii)   introducing into said cells a DNA molecule encoding an Fc receptor, and
    iii) reintroducing said cells into said mammal under conditions such that said DNA molecule is expressed and said Fc receptor thereby produced and the phagocytic potential of said cells thereby increased.

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15. The method according to claim 14 wherein said cells are T cells or B cells.

16. The method according to claim 14 further comprising contacting said cells with an effective amount of a drug that increases the phagocytic potential of said cells.

17. The method according to claim 16 wherein said drug is γ-interferon, an estrogen or estrogen analog, M-CSF or GM-CSF.

18. A liposome comprising a DNA molecule encoding an Fc receptor.

19. A bacterium comprising a DNA molecule encoding an Fc receptor.

20. A T cell comprising an exogenous DNA sequence encoding an Fc receptor.

21. A B cell comprising an exogenous DNA sequence encoding an Fc receptor.

22. A DNA construct encoding an Fc receptor comprising domains, or functional portions thereof, from at least two of FcγRI, FcγRII, the α chain of FcγRIII and the γ chain of FcγRIII, wherein said domains, or portions thereof, are such that said Fc receptor renders phagocytic a cell comprising same.

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23. The DNA construct according to claim 22
wherein said construct encodes the extracellular domain
of the α chain of FcRγIIIA, the transmembrane domain of
the γ chain of FcγRIIIA or FcγRI and the cytoplasmic
domain of the γ chain of FcγRIIIA.

24. The DNA construct according to claim 22
wherein said construct comprises, in a cytoplasmic
domain encoding portion thereof, at least two sequences
encoding Y-X2-L, wherein X2 represents any two amino
acids.

25. The DNA construct according to claim 24
wherein X2 represents the amino acids of a Y-X2-L
sequence of the cytoplasmic domain of FcγRIIA or the γ
chain of FcγRIII.

26. A cell comprising the construct according to
claim 22.

27. An Fc receptor comprising domains, or
functional portions thereof, from at least two of
FcγRI, FcγRII, the α chain of FcγRIII and the γ chain
of FcγRIII, wherein said domains, or portions thereof,
are such that said Fc receptor renders phagocytic a
cell comprising same.

28. The receptor according to claim 27 wherein
said receptor comprises, in a cytoplasmic domain

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thereof, at least two copies of the sequence Y-X2-L,
wherein X2 represents any two amino acids.

29. The receptor according to claim 28 wherein X2
represents the amino acids of a Y-X2-L sequence of the
cytoplasmic domain of FcγRIIA or the γ chain of
FcγRIII.

30. An Fc receptor comprising, in a cytoplasmic
domain thereof, at least one copy of the sequence Y-X2-
L in excess of the corresponding naturally occurring
cytoplasmic domain, wherein X2 represents any two amino
acids.

31. The Fc receptor according to claim 30 wherein
X2 represents the amino acids of a Y-X2-L sequence of
the cytoplasmic domain of FcγRIIA or the γ chain of
FcγRIII.

32. A DNA sequence encoding the Fc receptor
according to claim 30.

33. An Fc receptor comprising, in a cytoplasmic
domain thereof, at least one repeat of a Y-X2-L
sequence, wherein X2 represents any two amino acids.

34. The Fc receptor according to claim 33 wherein
X2 represents the amino acids of a Y-X2-L sequence of
the cytoplasmic domain of FcγRIIA or the γ chain of
FcγRIII.

35.   An Fc receptor comprising, in a cytoplasmic
domain thereof, one or more repeats of an FcγRIIA or γ
chain FcγRIIIA or FcεRI cytoplasmic domain or portion
thereof.

36.   The receptor according to claim 35 wherein
said portion includes Y-X2-L, wherein X2 represents the
amino acids of a Y-X2-L sequence of the cytoplasmic
domain of FcγRIIA or the γ chain of FcγRIII or of
FcεRI.

37.   A method of treating an infection comprising
administering to a mammal in need of such treatment a
DNA molecule encoding an Fc receptor,
      wherein said administration is effected under
conditions such that said DNA molecule is expressed in
the cells of said mammal, said Fc receptor is produced,
and the phagocytic potential of said cells thereby
increased, and
      wherein said cells phagocytose particles causing
said infection.

38.   The method according to claim 37 wherein said
infection is present in the lungs of said mammal and
said cells are lung cells.

39.   The method according to claim 37 wherein said
cells are liver cells.

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40.   The method according to claim 37 wherein said
cells are spleen cells.

41.   The method according to claim 37 wherein said
cells are T cells or B cells.

42.   The method according to claim 37 further
comprising administering to said mammal a drug that
increases the phagocytic potential of said cells.

43.   The method according to claim 42 wherein said
drug is γ-interferon, an estrogen or estrogen analog,
M-CSF or GM-CSF.

44.   A method of stimulating the phagocytic
potential of a cell comprising introducing into said
cell a construct comprising a nucleotide sequence
encoding the Syk gene under conditions such that said
Syk gene is expressed and phosphorylation of Fcγ
receptors in said cell is effected.

45.   The method according to claim 44 wherein the
phagocytic potential of said cell derives from a
naturally occurring Fcγ receptor.

46.   The method according to claim 44 further
comprising introducing into said cell a construct
comprising a sequence encoding an Fcγ receptor.

Fig. 1B

B. Phosphotyrosine induction
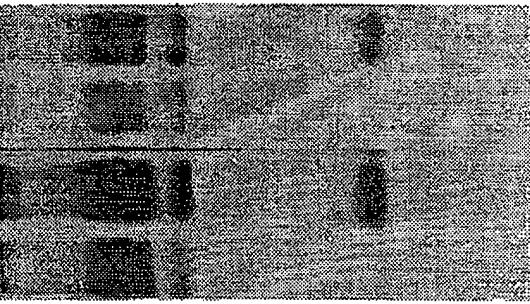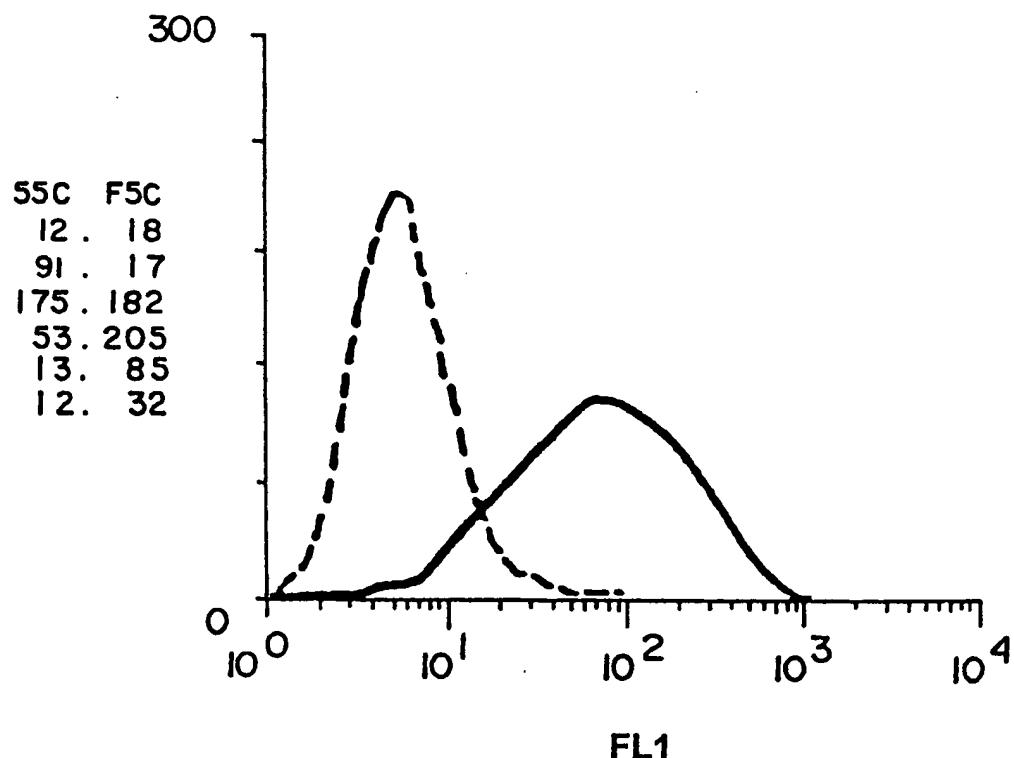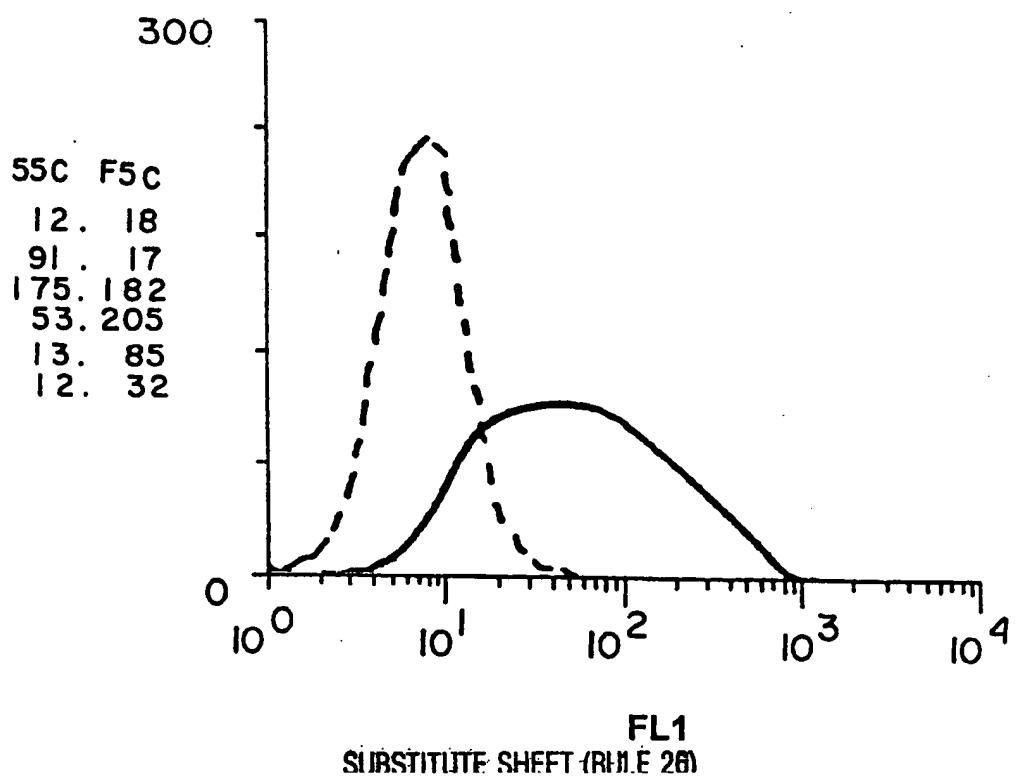
FcγRIIA
40 kDa

	SRC⁺	SRC⁻
EA:	IIA	IIA
	− +	− +

MW (kDa)
68 -
45 -

5 6   7 8

Fig. 1A

A. Biotinylation of membrane

FcγRIIA
40 kDa

	SRC⁺		SRC⁻	
	SHAM	IIA	SHAM	IIA

MW (kDa)
68 -
45 -

1 2   3 4

Fig. 2A

55C	F5C
12.	18
91.	17
175.	182
53.	205
13.	85
12.	32

FL1

Fig. 2B

55C	F5c
12.	18
91.	17
175.	182
53.	205
13.	85
12.	32

FL1

Fig. 3B

SRC⁻

SHAM | IIA
RAM | SRC | RAM | IV.3 | IV.3+SRC | IV.3+FYN

7 8 9 10 11 12

FcγRIIA
40 kDa

Fig. 3A

SRC⁺

Cell line:
Transfectant: SHAM | IIA
RAM | SRC | RAM | IV.3 | IV.3+SRC | IV.3+FYN

Antibody:

MW (kDa)
97 -
68 -
45 -

1 2 3 4 5 6

Fig. 4

Fig. 5

Fig. 6

HALF TIME OF RED
CELL CLEARANCE (HR)

Controls

Patients without Infection

Patients with Infection

Fig. 7

Fig. 8

Fig. 9

Fig. 10

J32/Fcγ RIIA

Fig. 11A

Control   IV.3

10^0 10^1 10^2 10^3 10^4

FL1

J32 - 3.2/Fcγ RIIA

Fig. 11B

Control

IV.3

10^0 10^1 10^2 10^3 10^4

FL1

SUBSTITUTE SHEET (RULE 26)
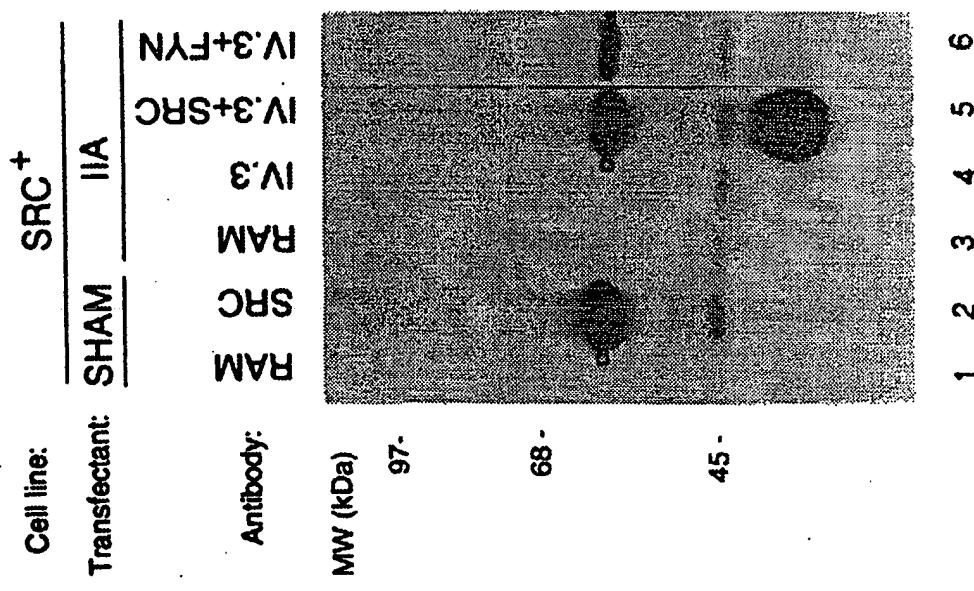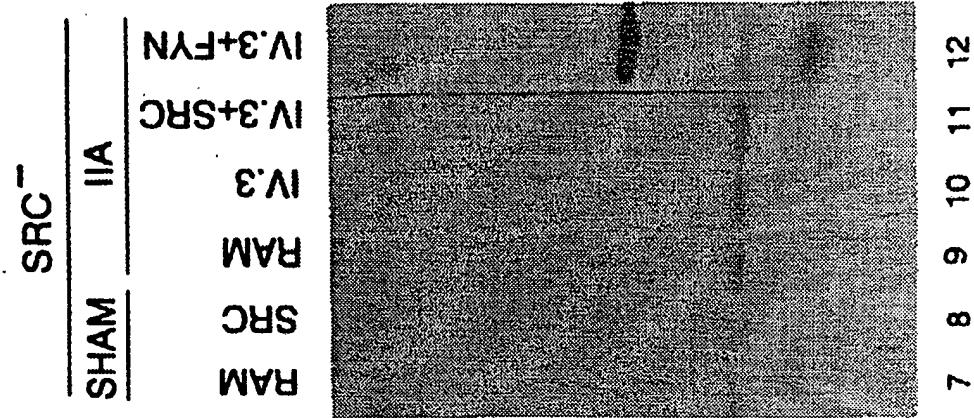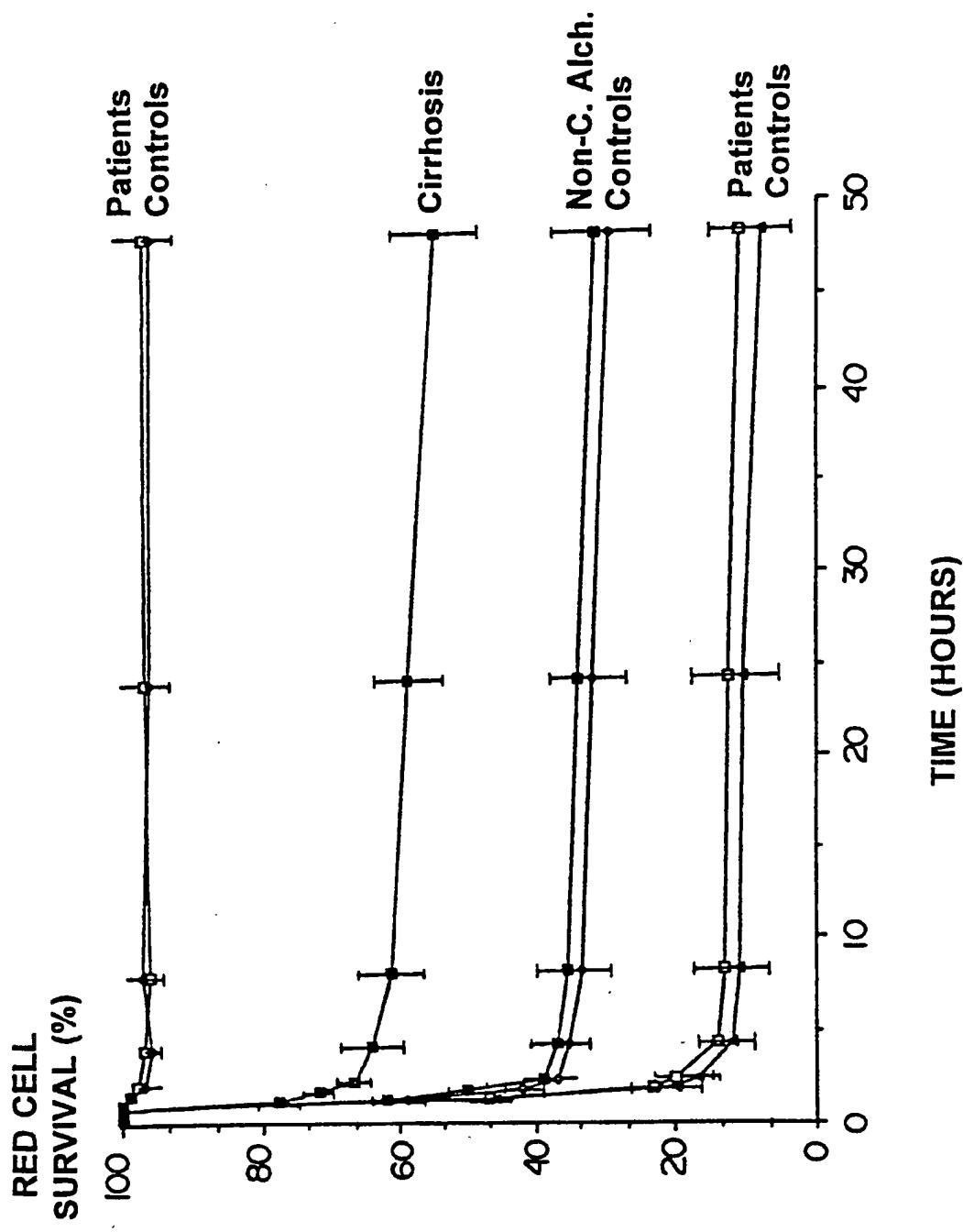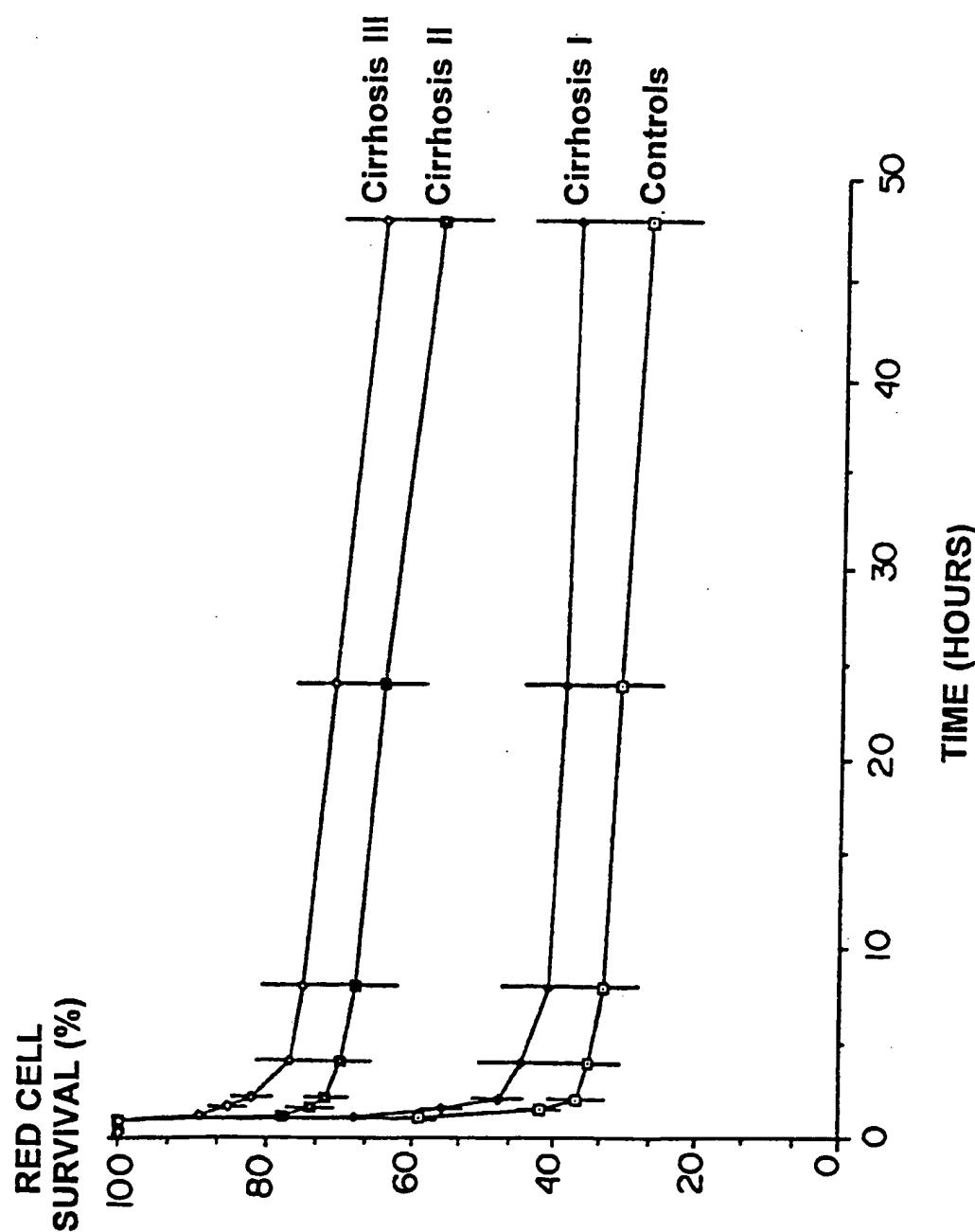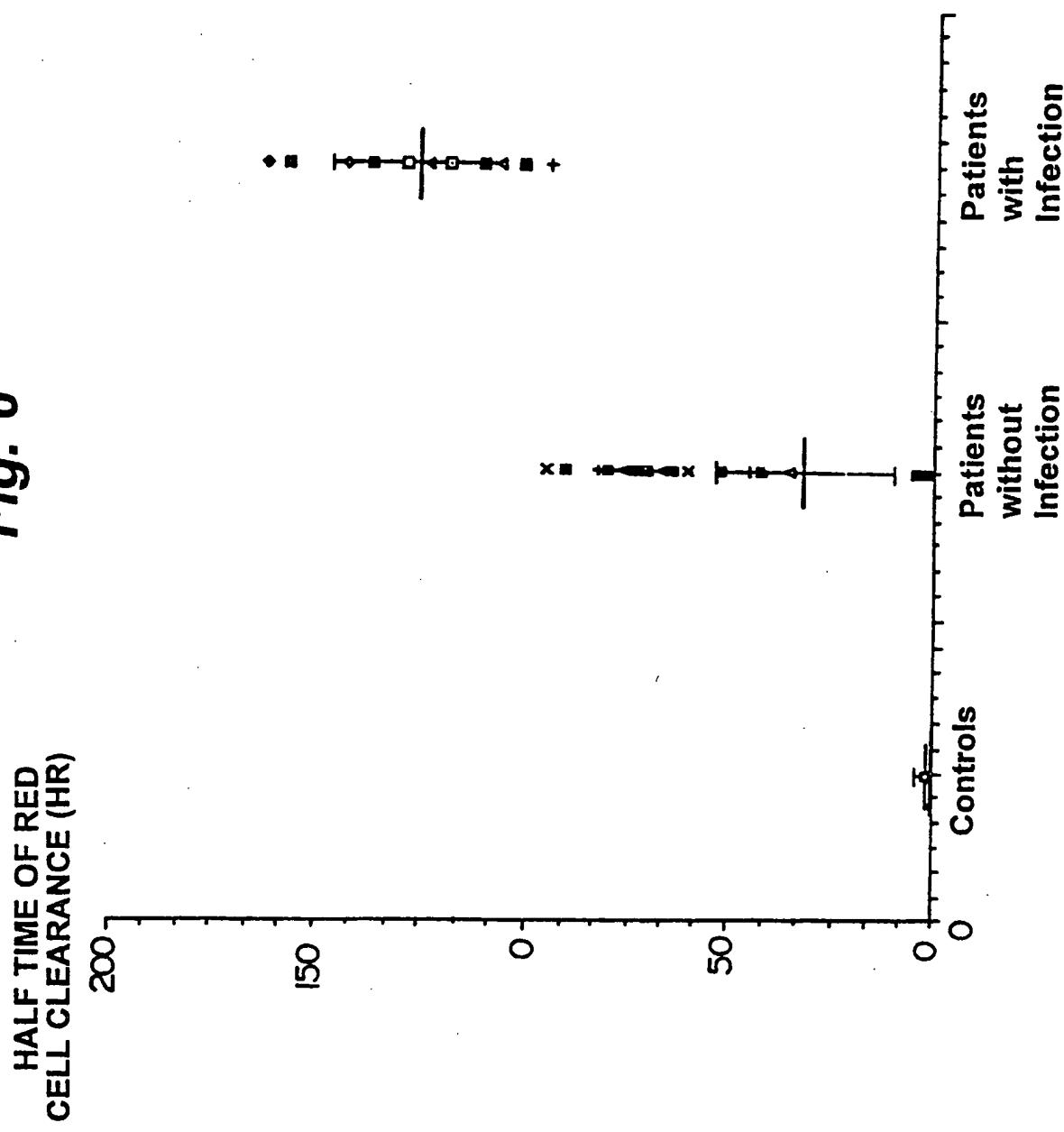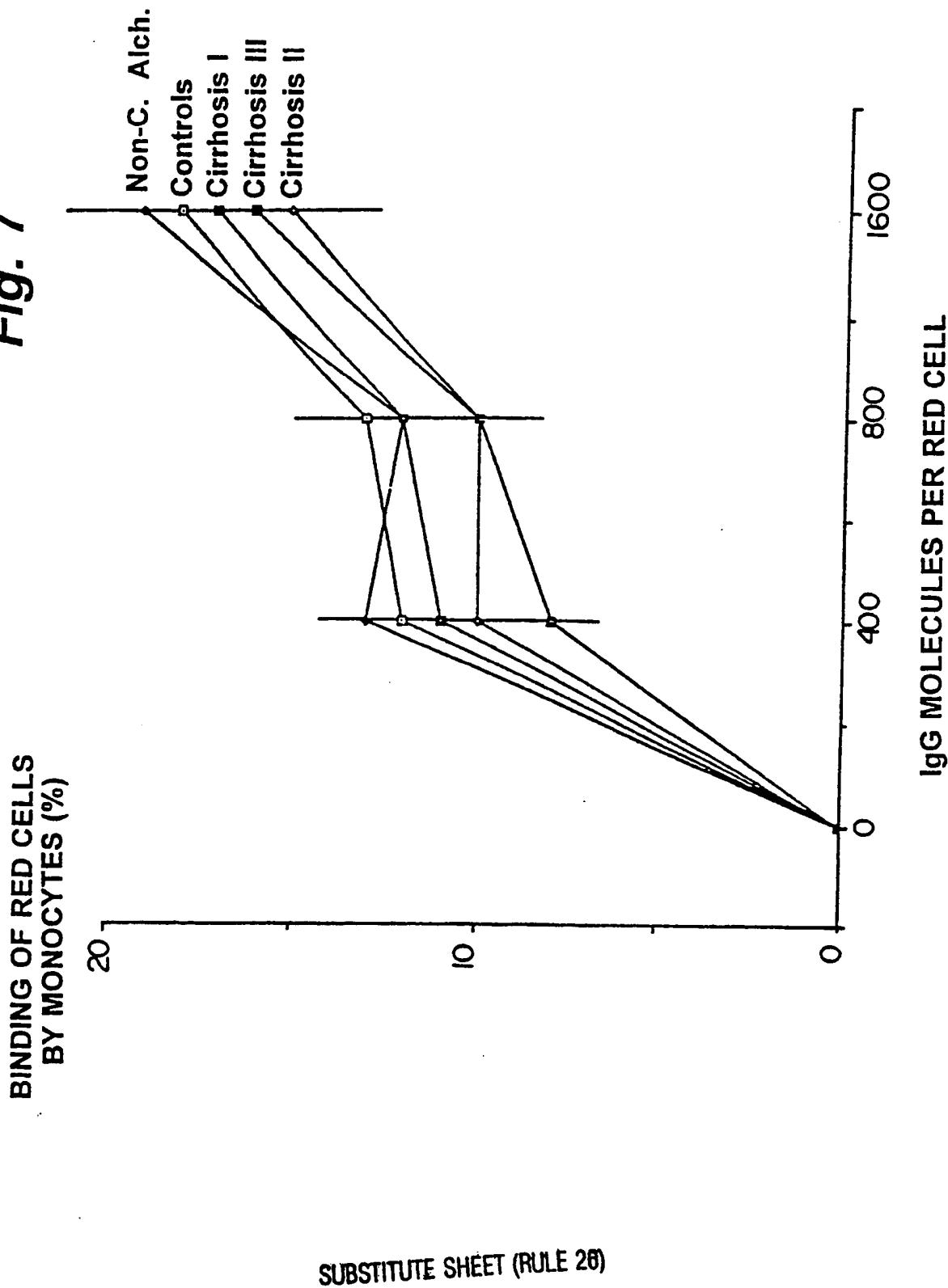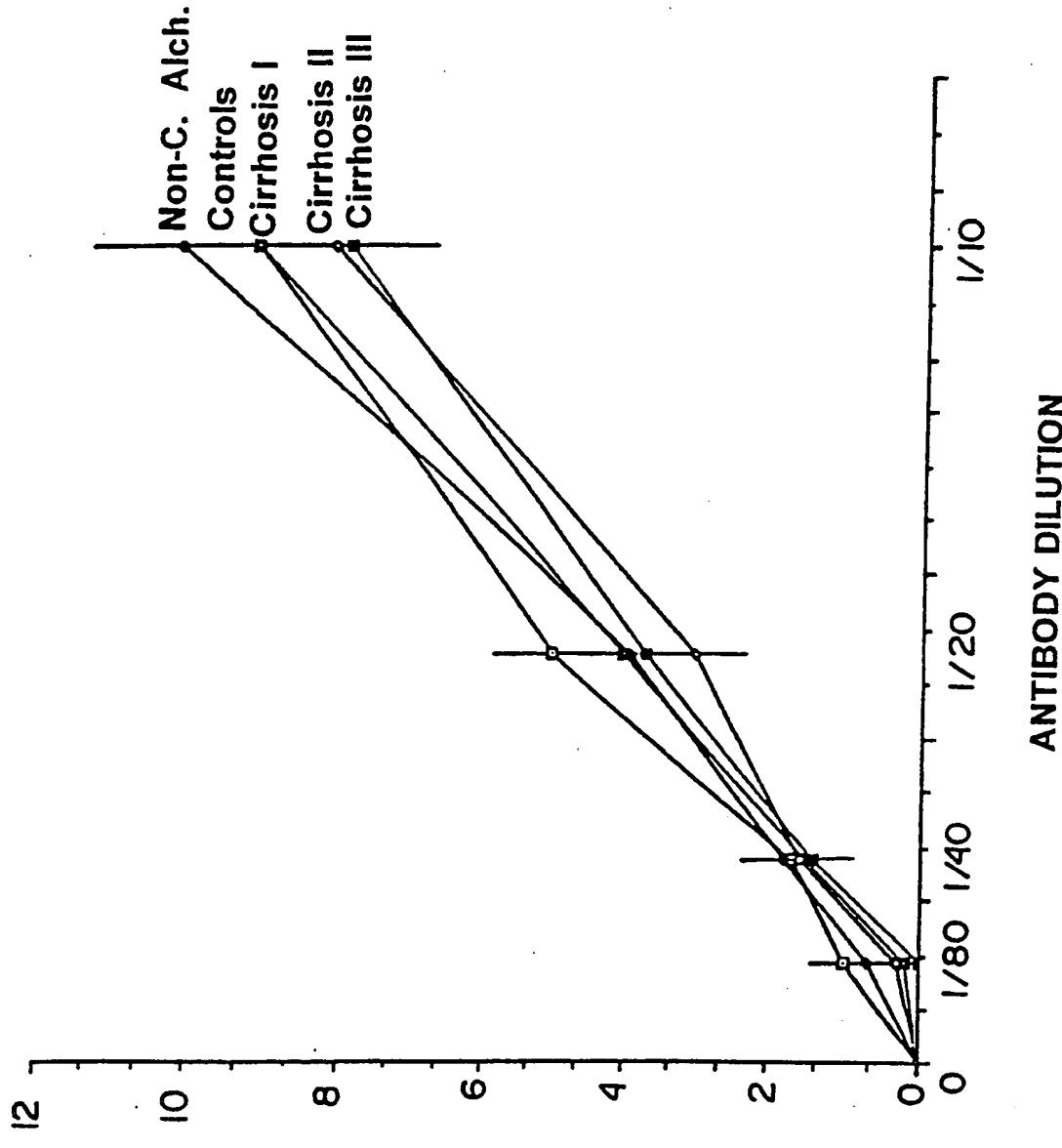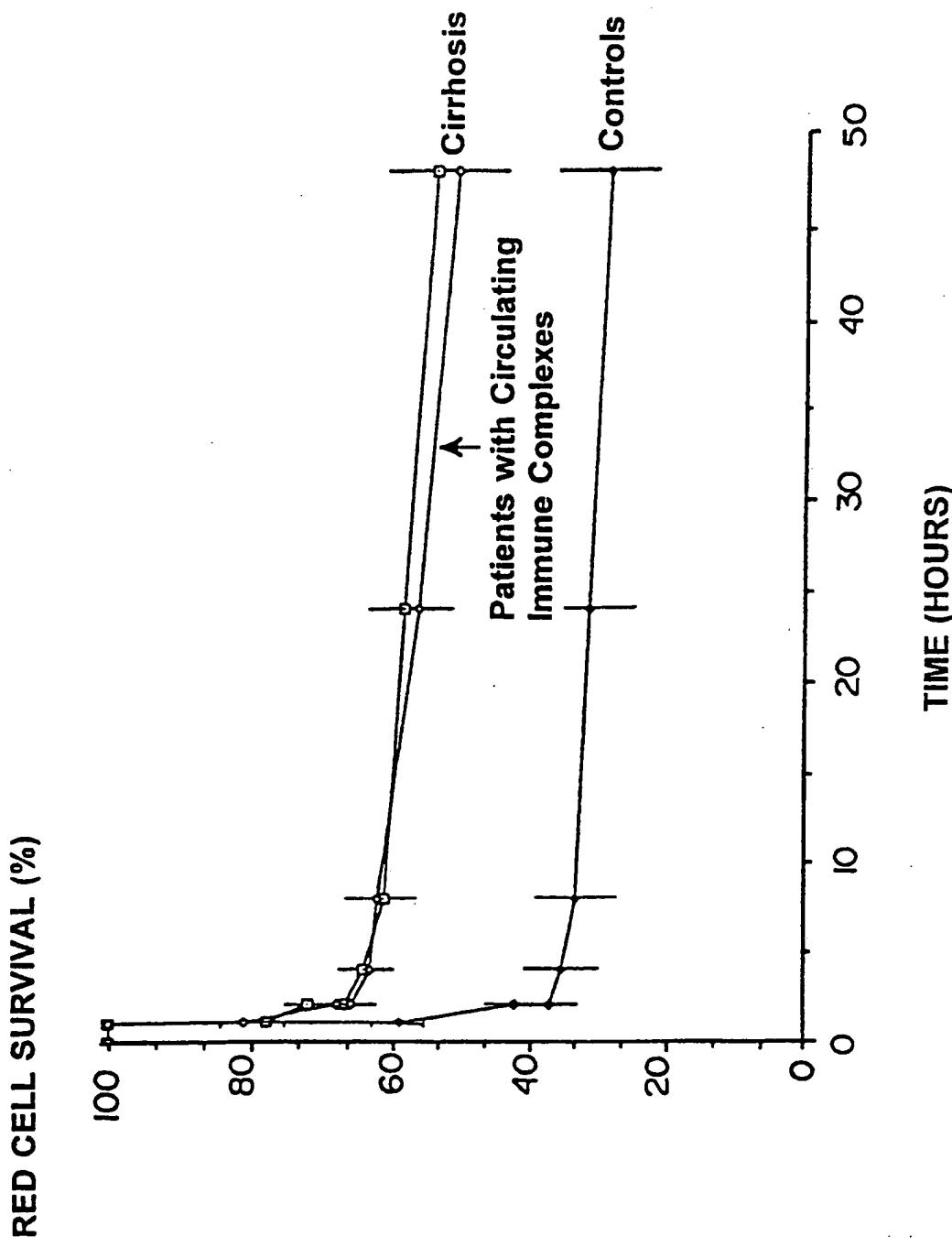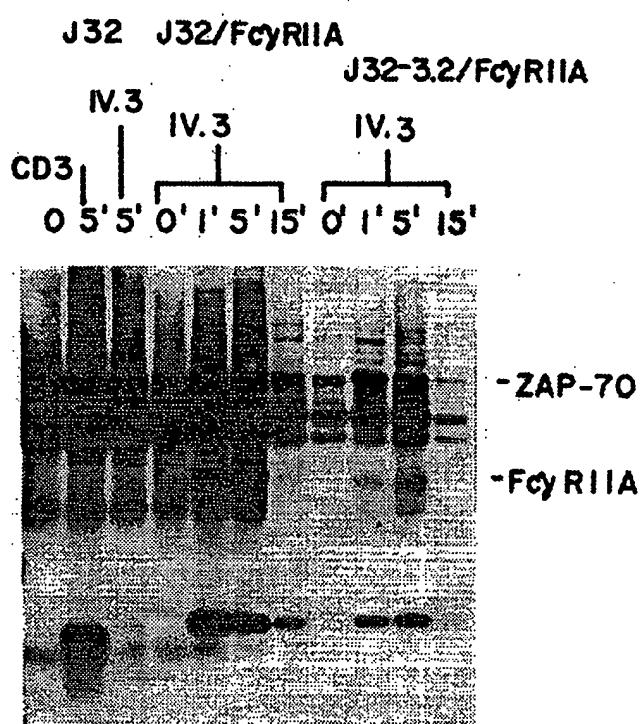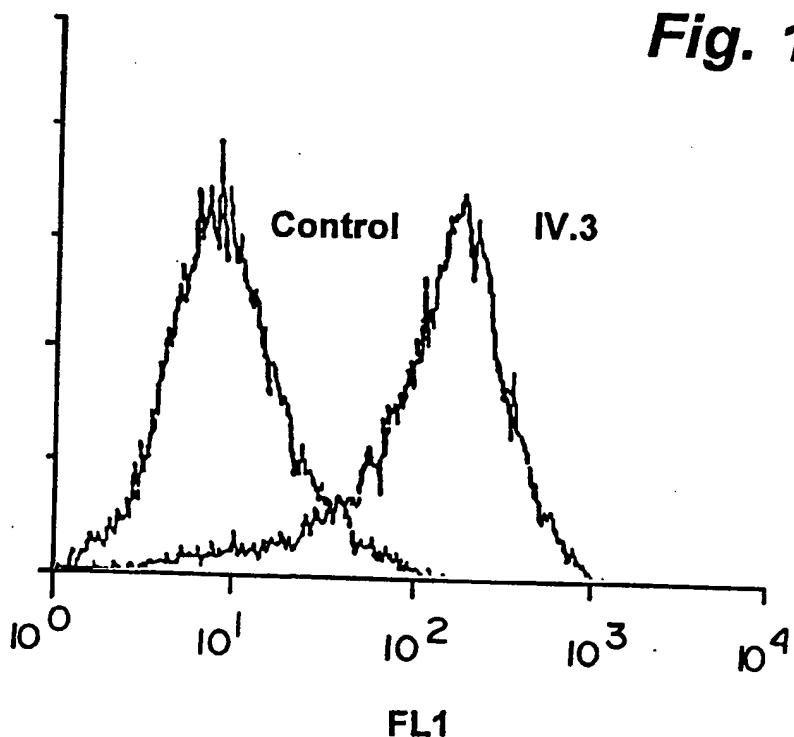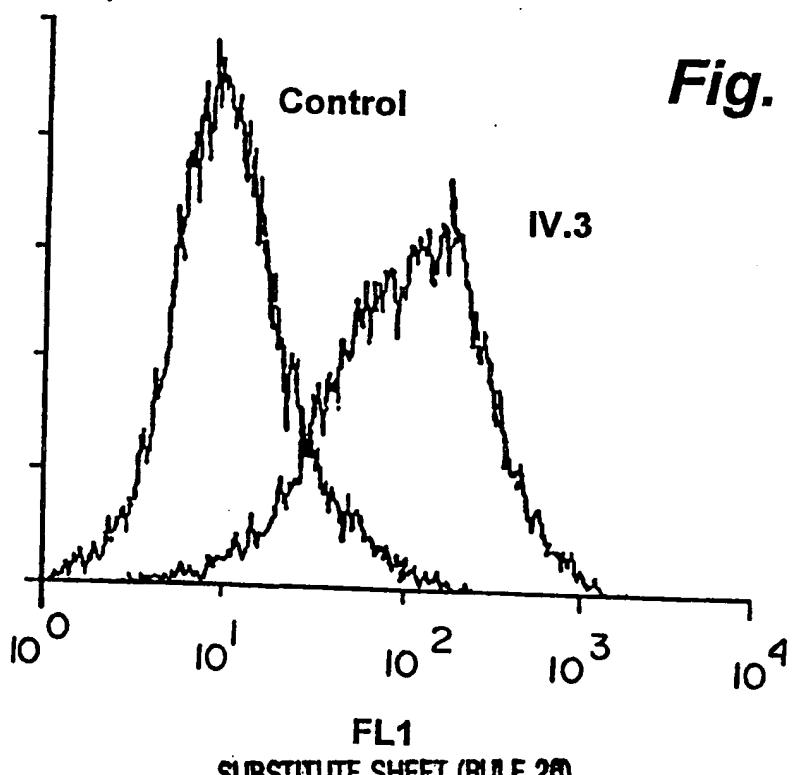
Fig. 12A

J32 / Fcγ-RIIA

Fig. 12B

J32-3.2 / Fcγ-RIIA
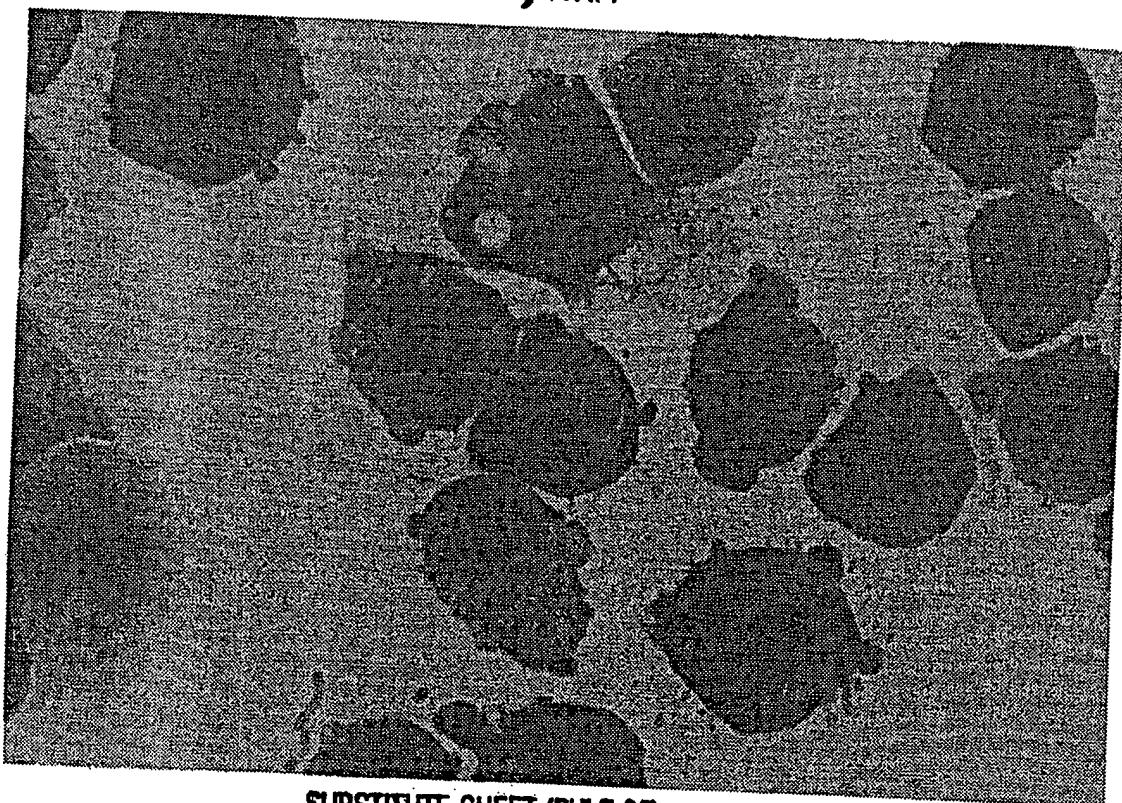
A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :A61K 48/00; C12N 15/00; C07K 14/00; C07H 21/00
US CL   :514/44; 435/172.3; 530/350; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S.   :   514/44; 435/172.3; 530/350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG
search terms: fc receptor, phagocytic potential, liposome, Y-X2-L, syk

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 244, issued 16 June 1989, Friedmann, "Progress toward human gene therapy", pages 1275-1280, see entire article.	10, 18
Y	Journal of Clinical Investigation, Volume 75, issued January 1985, Friedman et al., "Effect of estradiol and steroid analogues on the clearance of immunoglobulin G-coated erythrocytes", pages 162-167, see entire article.	1, 5, 6, 8, 12, 13, 37, 42 and 43
Y	Journal of Clinical Investigation, Volume 88, issued November 1991, Indik et al., "Human FcγRII, in the absence of other Fcγ receptors, mediates a phagocytic signal", pages 1766-1771, see entire article.	1, 5, 6, 8, 12-17, 20, 21, 37, 42 and 43

X	Further documents are listed in the continuation of Box C.	☐	See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 JANUARY 1995	JAN 2 5 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	SUZANNE ZISKA, PH.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

	C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 226, issued 26 October 1984, Anderson, "Prospects for human gene therapy", pages 401-409, see entire article.	9, 11, 19, 38 and 39
Y	GB, A, 2,223,755 (EVANS) 18 April 1990, see entire document.	9, 11, 19, 38 and 39
Y	Nature, Volume 358, issued 23 July 1992, Amigorena et al., "Tyrosine-containing motif that transduces cell activation signals also determines internalization and antigen presentation via type III receptors for IgG", pages 337-341, see entire article.	7, 40 and 41
Y	Cancer Research, Volume 51, issued 15 February 1991, Munn et al., "Role of low-affinity Fc receptors in antibody-dependent tumor cell phagocytosis by human monocyte-derived macrophages", pages 1117-1123, see entire article.	2-4, 22-26, 27-31, 32 and 33-36
Y, P	Biochemical and Biophysical Research Communications, Volume 200, No. 1, issued 15 April 1994, Yagi et al., " Cloning of the cDNA for the deleted SYK kinase homologous to ZAP-70 from human basophilic leukemia cell line (KU812)", pages 29-34, see entire article.	44-46

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

    Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                             ☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I. Claims 1-13, 18, 19, 22-26, 32 and 37-43, drawn to a method of increasing the phagocytic potential of cells present in a mammal comprising introducing into said cells a DNA molecule coding for an Fc receptor, classified in Class 514, subclass 44, for example, and drawn to a DNA construct encoding an Fc receptor and a cell comprising the construct, classified in Class 536, subclass 23.1, for example.

Group II.   Claims 14-17, 20 and 21, drawn to a method of increasing the phagocytic potential of cells of a mammal, classified in Class 424, subclass 93.21, for example.

Group III.   Claims 27-31, 33-36, drawn to an Fc receptor comprising domains, classified in Class 530, subclass 350, for example.

Group IV.   Claims 44-46, drawn to a method of increasing the phagocytic potential of a cell comprising introducing into said cell a construct comprising a nucleotide sequence encoding the Syk gene, classified in Class 514, subclass 44 or Class 435, subclass 172.3, for example.

        The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Invention II is drawn to the administration of cells to a mammal and involves different materials and procedures than does the method of Invention I or Invention IV. Invention III, drawn to the Fc receptor protein is a product not required for the practice of any of Inventions I, II or IV. Each grouping of claims forms a separate invention not linked to form a single inventive concept. PCT Rule 13 does not permit multiple distinct products and methods within a single application.